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AXIS STRAIN IN THEODOLITES, ITS EFFECTS, AND ONE METHOD OF REMOVAL[1]

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Abstract

Any deviation from geometrical form in close fitting theodolite axes, due to either manufacturing inaccuracies or subsequent dimensional changes in the metals composing them, produces strain in the axes which is transferred through the instrument, and, as the telescope is swung in azimuth, produces deflections of varying amount of the line of collimation. Permanent and variable changes may be produced, also, in the horizontal circle. A method of testing theodolites in the laboratory for the effect of axis strain is described in this paper, together with a method of removing its effect from the alidade and telescope axes of one make of theodolite.

Introduction

"It cannot be too strongly emphasized that a surveying instrument is merely an apparatus built around a line of collimation, and exists only for the purpose of setting that line in any desired direction and then measuring its setting. The introduction, therefore, of any device or part which is liable to deflect the line from its correct position must be carefully guarded against" (1).

An investigation in 1932 and 1933 by the authors of the present paper amply demonstrated the truth of the above quotation. In that investigation (3) it was shown that minute deviation from geometrical form existed in the steel cylindrical alidade and telescope bearings of a number of Wild Precision theodolites, owing probably to slight dimensional changes in the metal during the years following manufacture*. These imperfections of form produced changing strain in the steel axes as they revolved, and, through transfer of the strain through the instrument, caused changing deflections of the line of collimation which occasioned, in the measured values of angles, errors as large as two to four seconds of arc.

[1] *Manuscript received February 17, 1936.*
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* *The axes were surface hardened by heat treatment and in most cases subsequent warping occurred. It should be noted that a chemical hardening is now employed in which special steel is heated at a low temperature in ammonia gas; internal strain and consequent distortion are said to be practically non-existent. In addition, designs of alidade and telescope axes similar to those suggested by the authors in the original paper (3) are now employed by the maker for both sizes of theodolite manufactured by him.*

During that investigation a method of testing theodolites for the effect of axis strain was employed which consisted of the measurement of single and overlapping angles with the base of the theodolite rotated 120° after the completion of each third of a set. A least squares adjustment of the measured values permitted the calculation of the probable error of measurement of an angle. This probable error, when compared with a standard probable error representing the performance of an instrument with unstrained axes, indicated whether any axis strain existed.

This method of test had certain shortcomings. It was somewhat complex, and the adverse effect of axis strain on angular measurements was not easily apparent. It did not reveal the individual deflections of the line of collimation at various relations of the male and female alidade axes, and sometimes failed to reveal strain errors if a collimator was not pointed on at the azimuths at at which larger errors occurred. It was also impossible, from analysis of the results, to judge whether strain occurred in the telescope or alidade axis.

New Method of Testing for Axis Strain

To remedy these and other disadvantages a new method of testing theodolites for axis strain was devised. This method is similar in principle to that employed by Messrs. H. Wild to determine graduation errors (5). In general the method determines the relative deflections, due to axis strain, of the line of collimation at several equidistant azimuths.

By this method it is possible, by analysis of the observations, to judge whether strain exists in both alidade and telescope axes or only in the alidade. In addition the *relative* graduation errors at a limited number of points around the horizontal circle can be evaluated.

The test may be described as follows:

1. The marks sighted on are collimators. In the following tests the collimators employed were located in the instrument testing laboratory of the National Research Council, Ottawa (3, page 348).

2. Six sets of measurements of an angle of approximately 60° are made. In the first set the theodolite base (footscrews) is at a position A; between succeeding sets the base is rotated 60° anticlockwise, so that the six sets are taken with the base at positions A, $A-60°$, $A-120°$, - - - - - - - and $A-300°$ respectively.

The theodolite rests on a graduated ring which allows the whole instrument to be rotated any desired amount, as required by the above (See Fig. 1).

3. In each set a double measurement of the angle is made in each of six different positions of the horizontal circle. When the telescope is pointed on the left-hand collimator the six circle readings are 0°, 60°, 120°, 180°, 240° and 300°, respectively.

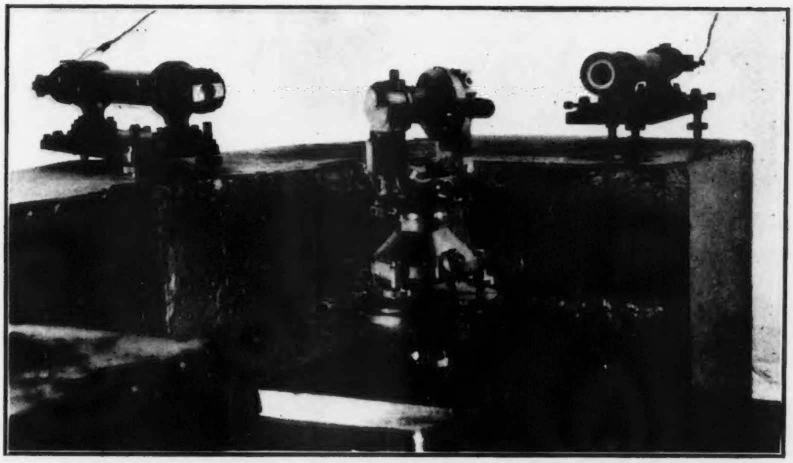

FIG. 1. *Equipment used for determining strain errors. The theodolite is supported on a heavy platform, adjustable for height, on which is mounted a turntable, permitting the whole instrument to be rotated and clamped in any desired azimuth. An upper plate on the turntable, with suitable vee grooves for the footscrews and threaded hole for adapters in the case of various instruments, can be moved laterally to centre the vertical rotation axis of the theodolite under a plummet or other indicator.*

4. Each double measurement (called a "position") consists of a clockwise swing of the alidade with telescope "direct", followed by an anticlockwise swing with telescope "reverse". The value of the angle for each position is calculated from the mean of the two swings.

5. At the end of the clockwise swing and beginning of the anticlockwise swing, the left-hand collimator is sighted on to check the action of the theodolite. The mean value of the readings on the left-hand collimator, two on each swing, is used in calculating the angle.

6. To eliminate the effect of any gradual change in the angular spacing of the collimators during a test program, the first three positions in each of the six sets are observed in order, followed by the remaining three positions in the reverse order of the sets.

7. In general the test occupies from three to four hours and, to avoid tiring of the observer with consequent loss of precision of observations, two observers participate, observing alternate positions. The one who is not observing, books the readings and computes the angles.

8. Although variable personal equation of pointing (2) enters into the measurements no attempt is made to eliminate it, as the conclusions reached are independent of it, provided that one observer makes all the measurements or two observers alternate as in Item 7 above.

General Case for Determination of Errors due to Axis Strain

The general case for determining the deflection of the line of collimation due to axis strain is as follows:—

Let S_θ be the deflection of the line of collimation due to axis strain when that line is at an angle θ in reference to the base of the theodolite,

α be the value of θ when the telescope is directed on the left-hand mark at the commencement of the test,

β be the angle between two marks, and

p be the error due to strain in the measured value of the angle β.

Then $S_{\alpha+\beta}$ is the deflection of the line of collimation when the telescope is pointed on the right-hand mark, and

$$p = S_{\alpha+\beta} - S_\alpha$$

In successive sets of measurements, the base of the theodolite being moved anticlockwise through the angle β between sets, the following relations hold—

$$\left.\begin{array}{lll} S_{\alpha+\beta} & - S_\alpha & = p_1 \\ S_{\alpha+2\beta} & - S_{\alpha+\beta} & = p_2 \\ S_{\alpha+3\beta} & - S_{\alpha+2\beta} & = p_3 \\ \text{-------} & & \\ S_{\alpha+(n-1)\beta} & - S_{\alpha+(n-2)\beta} & = p_{n-1} \\ S_{\alpha+n\beta} & - S_{\alpha+(n-1)\beta} & = p_n \end{array}\right\} \qquad (1)$$

If the angle β be of such value that $\beta = \frac{360°}{n}$

then $S_\alpha = S_{\alpha+n\beta}$

Then from Equation (1)

$$\left.\begin{array}{lllll} S_{\alpha+\beta} & - S_\alpha = p_1 & = r_1 & & \\ S_{\alpha+2\beta} & - S_\alpha = p_1 + p_2 & = r_2 & & \\ S_{\alpha+3\beta} & - S_\alpha = p_1 + p_2 + p_3 & = r_3 & & \\ \text{-------} & & & & \\ S_{\alpha+(n-1)\beta} & - S_\alpha = p_1 + p_2 + p_3 + \ldots\ldots + p_{n-1} & = r_{n-1} & & \\ S_\alpha & - S_\alpha = p_1 + p_2 + p_3 + \ldots\ldots + p_n & = r_n & = 0 & \end{array}\right\} \qquad (2)$$

Summing and dividing by n

$$-S_\alpha + \frac{S_\alpha + S_{\alpha+\beta} + S_{\alpha+2\beta} + \ldots\ldots + S_{\alpha+(n-1)\beta}}{n} = \frac{r_1 + r_2 + \ldots + r_{n-1}}{n} \qquad (3)$$

Letting $\dfrac{S_\alpha + S_{\alpha+\beta} + S_{\alpha+2\beta} + \ldots\ldots + S_{\alpha+(n-1)\beta}}{n} = A$

and $\dfrac{r_1 + r_2 + \ldots\ldots\ldots + r_{n-1}}{n} = C$

From Equations (2) and (3)

$$\left.\begin{array}{lll} S_\alpha & = & A^* - C \\ S_{\alpha+\beta} & = & A - C + r_1 \\ S_{\alpha+2\beta} & = & A - C + r_2 \end{array}\right\} \qquad (4)$$

etc.

* *For the maximum value of A see (d) page 97.*

or, relatively,

$$\left.\begin{aligned} S_{\alpha} &= -C \\ S_{\alpha+\beta} &= -C + r_1 \\ S_{\alpha+2\beta} &= -C + r_2 \end{aligned}\right\} \quad (5)$$

etc.

Application of General Case

The application of the general case to the test program described above is as follows:—

(a) The value of n is six, as the angle β is 60°.*

(b) From Equations (2), since $\Sigma p = 0$, it is evident that the mean value of the angle from the six sets is independent of strain errors, though it contains errors of observation.

(c) The values of p_1, p_2, etc. are the differences between the mean value of the angle and the values obtained from the six sets respectively. Subject to observational errors, they are the algebraic difference between the strain errors in two directions.

(d) The value of A in Equations (4) is the algebraic mean of the strain errors at six equidistant azimuths. In this investigation it is called zero, as its value does not interfere with the relation between the strain errors of any one instrument (see Equations (5)).

From more extended investigations of diametral graduation errors of circles by the same method, it is inferred that the value of A in the above formula is not greater than about one-third of the average value of the measured quantity (in this case the average diametral strain error) and may be safely neglected.

(e) In practice the test gives the *diametral strain error*, that is, the mean of two strain errors—one produced in the line of collimation when the telescope is "direct", the other when the telescope is "reverse" and the male axis has rotated 180° in the female alidade axis, the base of the theodolite having remained stationary.

Example of Test for Axis Strain

In Table I is given an example of the reduced readings obtained in a test of Wild Precision theodolite No. 309, employing the above program. Experience with this instrument in field work, as well as noticeable stiffness of the alidade axis, created a suspicion that considerable axis strain existed in this instrument.

* *It is obvious that other values of the angle β may be selected, such as 45°. The angle 60° has been chosen as it allowed a test to be completed in three to four hours, a period long enough for the highest precision in observing.*

TABLE I

TEST FOR AXIS STRAIN

Wild Precision Theodolite No. 309 (unremodelled)

Theodolite base	Sequence of readings	Circle reading on Collimator *A*	Observer	Coll. *A*	Coll. *B*	Coll. *A*	Theodolite base	Sequence of readings	Circle reading on Collimator *A*	Observer	Coll. *A*	Coll. *B*	Coll. *A*
				0° 00′	60° 00′	359° 59′					0° 00′	60° 00′	359° 59′
0°		0°	*M*	00″.0	02″.7	59″.9	−180°		0°	*F*	00″.0	01″.5	59″.9
	1	60	*F*		03″.4	59″.7		4	60	*M*		02″.3	59″.3
		120	*M*		04″.1	58″.7			120	*F*		04″.1	60″.6
		180	*F*		01″.6	60″.3			180	*M*		02″.7	60″.1
	12	240	*M*		03″.6	60″.3		9	240	*F*		02″.2	59″.6
		300	*F*		03″.5	59″.9			300	*M*		04″.0	58″.8
			*	00″.0	03″.15	59″.80				*	00″.0	02″.80	59″.72
			†	00″.0	03″.25					†	00″.0	02″.94	
−60°		0°	*F*		02″.3	60″.4	−240°		0°	*M*		02″.3	60″.8
	2	60	*M*		03″.3	60″.0		5	60	*F*		02″.8	59″.8
		120	*F*		04″.0	60″.6			120	*M*		04″.1	59″.3
		180	*M*		01″.8	60″.2			180	*F*		03″.5	60″.7
	11	240	*F*		02″.2	61″.1		8	240	*M*		04″.1	60″.6
		300	*M*		04″.4	60″.7			300	*F*		05″.2	60″.4
			*	00″.0	03″.00	60″.50				*	00″.0	03″.67	60″.27
			†	00″.0	02″.75					†	00″.0	03″.53	
−120°		0°	*M*		57″.6	60″.1	−300°		0°	*F*		01″.9	61″.1
	3	60	*F*		00″.9	60″.3		6	60	*M*		00″.7	60″.0
		120	*M*		59″.4	58″.1			120	*F*		02″.3	58″.8
		180	*F*		59″.8	59″.7			180	*M*		58″.6	59″.2
	10	240	*M*		59″.4	60″.0		7	240	*F*		00″.5	59″.7
		300	*F*		01″.7	59″.1			300	*M*		00″.5	59″.7
			*	00″.0	59″.80	59″.55				*	00″.0	00″.75	59″.75
			†	00″.0	00″.03					†	00″.0	00″.88	

* *Observed mean.* † *Mean corrected for closure.*

Calculation of Diametral Strain Errors

The calculation of the strain errors for theodolite No. 309, the result of a test of which is given in Table I, is shown in Table II.

TABLE II

CALCULATION OF DIAMETRAL STRAIN ERRORS

Wild Precision Theodolite No. 309 (unremodelled)

Theodolite base	Angle	Residual p	r	Diametral strain error
	60° 00′			
0°	03″.25	$+1''.02 = p_1$	$+1''.02 = r_1$	$-0''.55 = S_0$
60	02″.75	$+0''.52 = p_2$	$+1''.54 = r_2$	$+0''.47 = S_{60}$
120	00″.03	$-2''.20 = p_3$	$-0''.66 = r_3$	$+0''.99 = S_{120}$
180	02″.94	$+0''.71 = p_4$	$+0''.05 = r_4$	$-1''.21 = S_{180}$
240	03″.53	$+1''.30 = p_5$	$+1''.35 = r_5$	$-0''.50 = S_{240}$
300	00″.88	$-1''.35 = p_6$	$0 = r_6$	$+0''.80 = S_{300}$
	Mean 02″.23	Arith. mean 1″.18	Σr $+3''.30$; $\frac{\Sigma r}{n}$ $+0''.55 = C$	Arith. mean 0″.75

Standard of Comparison

While it is no doubt desirable, from the point of view of perfection, that strain errors in theodolite axes be zero, it is seldom found that this is the case. Any standard which may be established must take into account the precision of angular work desired, as well as the average performance of theodolites which have proved satisfactory for the class of work being performed.

Five interdependent standards are possible. If reference is made to Table II it is seen that these are:—

1. Average residual and
2. Maximum residual,
3. Average diametral strain error and
4. Maximum diametral strain error,
5. Maximum angular error indicated by a test, *i.e.*, the algebraic difference between the maximum plus and minus strain errors.

Any one of these five criteria may be employed. Except in limiting cases the authors use the *average diametral strain error*, which may be roughly defined as the average deflection of the line of collimation (telescope direct and reverse) at six equidistant azimuths. To transfer this criterion into other terms it is useful to remember that, from theoretical considerations, the average angular error from this source is less than one and a half times, and the maximum angular error about four times, the average diametral strain error.

Table III gives the average diametral strain error for nine Wild Precision theodolites which had been remodelled as shown in a previous paper (3).

TABLE III

DIAMETRAL STRAIN ERRORS (INCLUDING SMALL OBSERVATIONAL ERRORS) OF WILD PRECISION THEODOLITES (REMODELLED)

Theodolite No.	550	551	552	553	554	558	559	560	309*
Number of tests	2	3	1	1	2	1	1	2	1
Average diametral strain errors	0″.06	0″.06	0″.07	0″.08	0″.02	0″.09	0″.09	0″.12	0″.06

* *Tables I and II give the results of an axis strain test with this theodolite before its axes were remodelled. The average diametral strain error was then 0″.75.*

From Table III it may be judged that the average error of a direction due to axis strain (in addition to very small errors of pointing, etc.) is usually less than 0″.12. As, in Canadian primary triangulation, the average error of a direction derived from the adjustment of many nets is of the order of 0″.52 (4)†, and as the strain errors indicated in Table III are much less than this figure, they are probably sufficiently low for the highest class of angular measurement.

† *In this reference the* probable error *of a direction derived from the adjustment of the angle and side equations of 14 nets is given, the average being* $\pm 0''.44$. *From this the* average error *of a direction is deduced as* $0''.52$ $(\eta = 1.18r)$.

With the above considerations in mind, a *standard average diametral strain error of 0″.12 is employed** as one which can be reasonably met by primary triangulation theodolites.

Comparing the average strain error of Wild Precision theodolite No. 309 given in Table II (0″.75) with this standard (0″.12), it is easily seen that first order angular measurements cannot be expected with this instrument.

Source of Strain Errors

In the previous investigation (3) it was noted that strain was found to occur in both alidade and telescope axes of Wild Precision theodolites. An analysis of the present method of test allows conclusions to be drawn as to whether strain exists in both alidade and telescope axes, or only in the alidade axis. It is not possible with this test to state whether strain exists in the telescope axis unless it also exists in the alidade axis†.

First, a comparison of the average diametral strain error, calculated as in Table II, with the standard (0″.12) indicates whether excessive strain exists in the axis system. If so it may originate in both alidade and telescope axes or only in the alidade axis. A further analysis will show whether the telescope axis contributes to the strain effect.

Consideration of the relative positions of the male and female alidade axis, together with the position of the telescope, shows that, when the base of the theodolite, to which the female alidade axis is fixed, is at position 0° with telescope *direct* (or *reverse*), the male and female alidade axes are in the same relation as when the base is at 180° with telescope *reverse* (or *direct*). Thus if there is no strain in the telescope axis, the measured values of the angle when the base is at positions 0°, 60° and 120° should agree with those when the base is at 180°, 240° and 300° respectively, within the limits of the accidental errors.

An analysis was made of the fourteen tests of nine theodolites mentioned in Table III (the figures are not given in Table III). The average difference in the value of the angle as above, *viz.*, angle with base 0° minus angle with base 180°, etc., disregarding the signs of the differences, was found to be 0″.12 with a maximum of 0″.23.

If the average difference as above is less than 0″.12 it may be said that there is negligible strain in the telescope axis; if it is consistently greater than 0″.12, telescope axis strain of excessive proportions is present.

To exemplify the above analyses two tests may be considered.

* *In the original paper (3) a probable error of an angle measured in 16 positions of ±0″.10 was the standard employed. This corresponds to an average error of a direction measured in six positions of 0″.14, so the standards of the two tests (0″.12 and 0″.14), selected independently on the basis of the performance of instruments largely free from axis strain, are very nearly the same.*

† *See page 109.*

Strain in Both Alidade and Telescope Axes

In the test of No. 309, the result of which is shown in Tables I and II, the average diametral strain error (0″.75) when compared with the standard strain error (0″.12) indicates that axis strain existed to an undesirable degree in this instrument.

The differences in the angles shown in Table II when the base was at 0°, 60° and 120° and when it was at 180°, 240° and 300° are 0″.31, 0″.78 and 0″.85 respectively, an average of 0″.65. This is so much greater than 0″.12 mentioned as being employed as a standard that it is evident that serious strain existed in the telescope axis of this theodolite, as well as in the alidade axis. Confirmation of this was given on dismantling the telescope axis. Fig. 2 shows the journal carrying the vertical circle end of the telescope. This journal showed an uneven burnished mark which very definitely indicated a lack of alignment of the blocks at the tops of the standards, which, considering the small tolerance of the cylindrical bearings, would produce a variable strain or reversing flexure in the telescope axis as it revolved, and could scarcely fail to cause errors when points at different elevations were sighted on. *The fact that this obvious strain did not produce a very noticeable stiffness in the movement of the telescope is a dangerous feature of this condition.*

FIG. 2. *One end-bearing of the telescope axis of theodolite No. 309. Near the upper end may be seen the uneven burnished mark confirming the test indications that the bearing and the telescope axis (which envelopes it) were not in accurate alignment.*

TABLE IV

TEST INDICATING STRAIN IN ALIDADE AXIS BUT NONE IN TELESCOPE AXIS

Wild Precision Theodolite No. 559

Base	Angle	Residual p	r	Diam. strain error
	59°-59′			
0°	56″.68	+0″.65	+0″.65	−0″.48
60	56″.11	+0″.08	+0″.73	+0″.17
120	55″.30	−0″.73	0	+0″.25
180	56″.81	+0″.78	+0″.78	−0″.48
240	56″.01	−0″.02	+0″.76	+0″.30
300	55″.25	−0″.78	−0″.02	+0″.28
Mean	56″.03	0″.51	+0″.48	0″.33

Strain in Alidade Axis but None in the Telescope Axis

Table IV shows an example of this condition.

The standard for average diametral strain error (0″.12) compared with that of No. 559 (0″.33) indicates that strain to a serious degree existed in the axis system of this theodolite.

However, the differences in the angles shown in Table IV when the base was at 0°, 60° and 120° and when it

was at 180°, 240° and 300° are 0″.13, 0″.10 and 0″.05 respectively, an average of 0″.09. Since this figure is less than the standard 0″.12 the conclusion was that negligible strain existed in the telescope axis. The trouble was hence localized in the alidade axis.

The axes of this theodolite had been remodelled, and a test 12 months before had shown no axis strain (Table X in (3)). Following the test the result of which is shown in Table IV, an examination indicated that the lower bearing of the female alidade axis had become slightly oval. This was lapped, after which the instrument was again tested, giving the satisfactory results shown in Table III. The conclusion is that further change in the shape of the lower bearing of the alidade axis occurred during the 12 months following the remodelling. This conclusion somewhat confirms the statement in the paper referred to (3, p. 356), that "a better design (for the lower bearing of the alidade axis) might be two solid pads placed 120° apart, with a third spring supported pad."

Several Causes of Apparent Alidade Axis Strain

So far it has been assumed that alidade axis strain is caused only by a deviation from geometrical form of the alidade axis itself (male or female). There are, however, a number of associated troubles which give the same effect as geometrical deformation of the alidade axis; the presence of these troubles is clearly revealed by the test for axis strain, though the discovery of their cause is frequently a very puzzling problem.

The horizontal clamp arm is frequently a source of trouble. Owing to various causes this may develop uneven friction as the telescope is revolved in azimuth; the application of the clamp may distort the theodolite base, etc.; in all such cases the test apparently indicates excessive alidade axis strain, whereas the trouble may be in the clamp.

Again, if some part of the alidade touches some part of the base of the theodolite, except at the bearing surfaces of the axis, or if some part of the male axis or its connections momentarily or lightly touches some part of the female axis which it should not, even though the contact cannot be detected by increased or uneven friction, it will be revealed by the test as excessive alidade axis strain.

A further possible cause of apparent alidade axis strain is deformation, with consequent strain, in the axis fitting of the horizontal circle. With the original alidade axis of the Wild theodolite any strain in the circle axis is communicated to the alidade axis, and would be indicated in the test as changing alidade axis strain as the circle is revolved. With the remodelled alidade axis (3) the probability of the transmission of strain from the circle axis to the alidade axis is greatly reduced.

Testing of Small Theodolites for Axis Strain

Many makes of 5- and 6-in. theodolites can be read directly to five seconds and by estimation to one second; the precision of circle reading is sufficient to measure the effect of axis strain. At least three makes of smaller theodo-

lites are available in which the circles can be read to one second—Wild Universal, Zeiss and Cooke "Tavistock". The Wild and Zeiss have cylindrical steel alidade and telescope axes of much the same type as those of the Wild Precision theodolite, in several of which axis strain of disturbing proportions had been detected and removed (3). Most of the older types of small micrometer theodolites, as well as the Cooke "Tavistock", have conical alidade axes and the telescope axis rests in Y's.

It seemed possible that axis strain errors might also affect angular measurements made with these very desirable types, particularly those with cylindrical axes. While many field experiences indicated that errors of large proportions would be rare, one Wild Universal theodolite produced angular errors in the field of the order of five seconds, much in excess of those which were expected of it. It was then decided to subject a number of these small theodolites to the test for axis strain described above.

It was not feasible to obtain a sufficiently large number of the various makes which were old enough to have developed warping subsequent to manufacture, except in one instance (the Wild Universal). No general inferences can therefore be made from the following tests in regard to any makes other than this one.

As an example of the worst case of axis strain discovered, Table V gives a synopsis of the mean of three tests given Wild Universal theodolite No. 556, which was purchased in 1926.

This test clearly indicates that a maximum angular error of the order of 4″.8 (2″.51 + 2″.26) due to axis strain was possible with this theodolite, and, judged by the standards employed for larger theodolites, that both telescope and alidade axes contributed to the error. The very dangerous feature of the condition of this instrument was that, while there was a slight feeling of stiffness in the movement of the alidade axis, there was little appreciable stiffness in the movement of the telescope, and the instrument was (before the test) deemed to be in condition to be sent to the field to execute secondary triangulation in which angular precision of the order of one second was required.

TABLE V

SYNOPSIS OF TEST FOR STRAIN ERROR—WILD UNIVERSAL THEODOLITE No. 556 (UNREMODELLED)

(Mean of three tests)

Base	Angle	Residual p	r	Diametral strain error
	60° 00′			
0°	06″.23	+2″.94	+2″.94	−0″.43
60	00″.16	−3″.13	−0″.19	+2″.51
120	04″.03	+0″.74	+0″.55	−0″.62
180	03″.86	+0″.57	+1″.12	+0″.12
240	00″.34	−2″.95	−1″.83	+0″.69
300	05″.13	+1″.84	+0″.01	−2″.26
Mean	03″.29	2″.03	+0″.43	1″.10

Table VI gives the average diametral strain errors of several of these small theodolites.

TABLE VI

AVERAGE DIAMETRAL STRAIN ERRORS WITH SMALL MODEL THEODOLITES
(Unremodelled axes)

Theodolite		Average diametral strain error	Theodolite		Average diametral strain error
Wild Universal	2106	0″.04	Wild Universal	1889	0″.52
Wild Universal	555	0″.27	Watts 5 in. Micrometer (Cylindrical axes)	6960	0″.31
Wild Universal	556	1″.10	Zeiss 3-in.	16566	0″.28
Wild Universal	557	0″.72	Cooke Tavistock (small size, new instrument)		0″.04
Wild Universal	1712	0″.38			

If the values in Table VI are compared with the standard strain error (0″.12) for first order theodolites, it is seen that two of these small theodolites meet this standard. None of the others approach it. It will be shown in Table VIII that the same standard is applicable to these small theodolites, though it is not necessary that so high a standard be required for them.

Outstanding Examples of Tests of Theodolites with Strained and Unstrained Axes

As a comparison of average strain errors may not give an adequate appreciation of the possible harmful effects of axis strain on angular measurements, there are collected in Table VII outstanding examples of angular measurements with theodolites having strained and unstrained axes. These examples were obtained during the tests described in this paper.

TABLE VII

SUMMARIES OF TESTS WITH THEODOLITES WITH STRAINED AND UNSTRAINED AXIS SYSTEMS

Theod. base	Theodolites with strained axes		Theodolites with unstrained axes	
	Wild Precision theod. no. 309 (unremodelled)	Wild Universal theod. no. 556 (unremodelled)	Wild Precision theod. no. 554 (remodelled)	Wild Universal theod. no. 2106 (unremodelled)
	Angle (see Tables I and II)	Angle (see Table V)	Angle	Angle
	60° 00′	60° 00′	60° 00′	60° 00′
0°	03″.25	06″.23	06″.28	01″.99
60	02″.75	00″.16	06″.30	01″.92
120	00″.03	04″.03	06″.28	01″.99
180	02″.94	03″.86	06″.30	02″.00
240	03″.53	00″.34	06″.27	02″.01
300	00″.88	05″.13	06″.29	02″.09
Average diametral strain error	0″.75	1″.10	0″.01	0″.04

A comparison of the range of the angles with each of the different instruments tells the tale of the effect of axis strain without further elaboration.

Removing Axis Strain

As Wild Universal theodolites are light and convenient in operation, and as both field and laboratory experience indicated they should be capable of high precision, it was desirable that a means be found for eliminating the indicated strain errors in instruments already manufactured. Accordingly both alidade and telescope axes were remodelled as shown in Figs. 3 and 4.

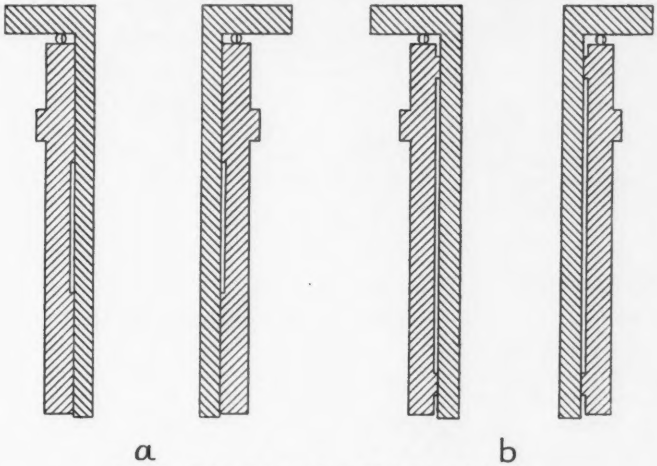

FIG. 3. *Original (a) and modified (b) alidade axes.*

In the alidade axis the upper and lower cylindrical bearings were each reduced to 3 mm. in width, care being taken to leave unchanged that part of the bearing which high polishing of the steel showed to be tightest. This tightness was later reduced by lapping to an extent which successive strain tests indicated as being necessary.

The end bearings of the telescope axis were remodelled in the same way as were those in the larger Wild (3); each bearing was reduced in width to about 3 mm., and a 60° sector was entirely removed from the top of the bearings. This practically made an inverted-Y bearing for the telescope axis.

There were three reasons for adopting a 3 mm. bearing at the top of the alidade axis in the Universal Wild theodolite, instead of the ball bearing in the 45° cone, as was used in remodelling the larger Wild. When the 45° cone is cut at the top of the female axis to provide a kinematic bearing, the outer hardened surface of the steel is cut away and the ball bearings run on softer metal, which is hence liable to become grooved and pitted. Again, the top of the female bearing of the Universal Wild is quite thin and would allow very little cutting for the 45° cone, thus necessitating very small balls. Lastly, this method does not require as high accuracy, and can be done in any good machine shop.

It is to be noted that the reduction in width of the bearing surfaces is liable to upset the strain equilibrium in the metal, with a consequent resumption of the warping process. Owing to the approach to line bearings, any warping

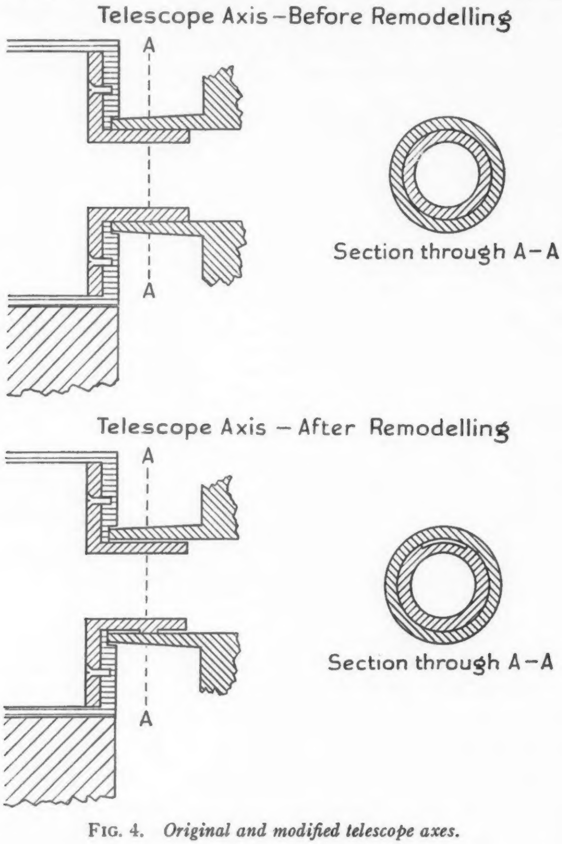

FIG. 4. *Original and modified telescope axes.*

in a direction parallel to the axis is of negligible consequence; experience has shown, however, that alteration in shape of the reduced cylinders is liable to occur, and this may result in strain errors, which are easily removed by a small amount of lapping of the reduced cylinders.

Table VIII gives the results of strain tests of four small theodolites with remodelled axes, together with those for two others.

Conclusion Regarding Small Types of Theodolites

A comparison of the results in Table VIII with those in Table III indicates that the precision of angular measurements with these small types of theodolites, when they are in perfect condition, is comparable with that of larger

geodetic models. Naturally the telescope power of the smaller models is less than that of the larger ones. This makes them less useful for cases in which the optimum telescope power is required, but the results of Table VIII indicate that these tiny theodolites are otherwise capable of angular measurements of the highest precision required in triangulation if the accuracy which is, presumably, incorporated in them at the time of manufacture is maintained.

TABLE VIII

TESTS WITH WILD UNIVERSAL AND OTHER THEODOLITES

Theodolite	Average diametral strain error
Wild Universal 544 (remodelled)	0″.05
Wild Universal 555 (remodelled)	0″.05
Wild Universal 556 (remodelled)	0″.14*
Wild Universal 557 (remodelled)	0″.09
†Wild Universal 2106	0″.04
†Cooke Tavistock (small size)	0″.04
	Mean 0.″07

* *The remodelled alidade axis of theodolite No. 556 had to be lapped to produce results as small as the above.*

† *Repeated from Table VI. These instruments were not remodelled.*

After the remodelling and testing described above, four of the remodelled Universal Wild theodolites were used on field work in very mountainous country in which transportation was by pack-horse and back packing. Results were quite satisfactory. Subsequently these instruments were dismantled and cleaned, and the axes examined for wear. They were then reassembled and tested for axis strain. No evidence of excessive wear was noted, but the tests indicated that in two of the instruments alidade axis strain had developed in the nine-month interval; this was traced to a slight tightness in the reduced cylinders of the female alidade axes. The conclusion is that slight further warping may be anticipated in axes following the remodelling described above, and that regular tests and possibly a small amount of lapping are required until warping ceases.

Strain in Horizontal Circle Axis

That the above-described tests showed strain in the cylindrical alidade and telescope axes of a number of earlier models of Wild theodolites, both Precision and Universal models, indicated the possibility that similar strain might be present in the similarly designed bearings in the axis carrying the horizontal circle. Any strain in this axis could have two types of adverse effects. As the circle is revolved there would be an alteration of the strain in the unremodelled alidade axis, though there should be very little strain transmitted through remodelled axes. Permanent and also changing distortion of the horizontal circle itself could also be caused by warping and strain in the circle axis.

Very strong suspicions were aroused during the tests previously described that circle axis strain actually did have the above-mentioned effects in various instruments, more particularly in the smaller types. Two conclusions became fairly clear, however, when all the evidence was collected:—

(i) The effect of circle axis strain was a minor cause of angular inaccuracy *with theodolites having remodelled alidade axes*, especially when a number of circle positions were employed.

(ii) Further tests would be necessary to secure that degree of proof which would warrant extended reference to the subject.

Circle Graduation Errors

The principle of the method of determining diametral strain errors given on pages 96 and 97 applies equally well to the determination of diametral graduation errors, *i.e.*, the average errors of pairs of graduations 180° apart.

In each of the six sets of the test program (Table I) there are six double measurements of the angle with circle settings of 0°, 60°, 120°, 180°, 240° and 300° respectively. The means of the sets of Table I are largely free from graduation errors (at least each set is similarly affected), but all the angles in each set are affected by a certain axis strain error. If we arrange the angles of Table I as shown in Table IX, in which each horizontal row contains the angle with the same circle setting from each of the six sets, each of the angles in any row is affected by the same graduation error, but the mean of the row is largely free from strain error.

TABLE IX

OBSERVATIONS IN TABLE I ARRANGED FOR CALCULATION OF GRADUATION ERRORS

Circle setting	Theodolite base						
	0°	60°	120°	180°	240°	300°	Mean
0°	02″.7*	02″.1	57″.6	01″.5	01″.9	01″.4	01″.20
60	03″.6	03″.3	00″.7	02″.7	02″.9	00″.7	02″.32
120	04″.7	03″.7	00″.4	03″.8	04″.5	02″.9	03″.33
180	01″.4	01″.7	59″.9	02″.7	03″.1	59″.0	01″.30
240	03″.5	01″.7	59″.4	02″.4	03″.8	00″.6	01″.90
300	03″.6	04″.0	02″.2	04″.6	05″.0	00″.7	03″.35

* *Readings are corrected for closure.*

TABLE X

CALCULATION OF DIAMETRAL GRADUATION ERRORS

Circle reading	Angle	Residual p	r	Diametral graduation error
0°	01″.25	−0″.98	−0″.98	+0″.69†
60	02″.11	−0″.12	−1″.10	−0″.29
120	03″.34	+1″.11	+0″.01	−0″.41
	02″.23		−0″.69	

† *From Equations (4) on page 96, which apply to this case, each of the diametral graduation errors of Table X should be increased algebraically by the quantity A, whose magnitude can be determined only by similar measurements of other angles of suitable size. The relative values of the diametral graduation errors in Table X are unaffected.*

For the possible magnitude of A see Item (d) page 97.

On the assumption that the diametral graduation errors at 0°, 60° and 120° are the same as those at 180°, 240° and 300° respectively (if changing distortion of the horizontal circle exists, the above assumption is not tenable), the measurements of Table IX are employed to reveal the diametral graduation errors as shown in Table X. Under the heading "Angle" is given the mean of angles at 0° and 180°, 60° and 240°, 120° and 300°.

Strain in Telescope Axis Alone Not Disclosed by This Test

A limitation of the strain test described in this paper is that strain in the telescope axis alone is not disclosed. It is only when accompanied by strain in the alidade axis that it can be shown to exist by this test.

As the collimators are on the same level, the telescope is stationary while rounds of observations are being made. Thus, if only telescope axis strain is present, it is *constant* during the observing of "direct" or "reverse" rounds and affects all angles alike. It is only when variable strain from the alidade axis is transferred through the standards, telescope axis and telescope to the line of collimation and changes any strain in the telescope axis as the telescope is swung in azimuth that it is revealed, as shown on page 100.

It is hence possible that telescope axis strain still exists in some of the theodolites which the above test shows to be free from strain, and that horizontal angles measured between objects at different angles of elevation will still be in error, owing to strain in the telescope axis.

The above-mentioned possibility can be eliminated only by the adoption of a telescope axis design which precludes the production of changing strain. The employment of the principles of kinematic design, as exemplified in Y-bearings, is one method, and as the remodelled telescope axis shown in Fig. 4 approaches an inverted Y-bearing there is considerably less likelihood of residual telescope axis strain with this design than with the original.

Observational and Other Errors

Very little has been said in this paper about accidental errors of observation, and no attempt has been made to separate them from instrumental errors.

It is certain that accidental errors of telescope pointing and micrometer reading are as small as the smallest instrumental errors it was intended to

TABLE XI

AVERAGE OBSERVATIONAL AND OTHER SMALL ERRORS

Theodolite and condition	Number of "positions"	Average error of mean diametral strain error
Wild Precision theodolite. (Negligible axis strain. Most suitable arrangement of diaphragm lines)	828	0″.01
Wild Precision theodolite. (Considerable axis strain. Medium suitable arrangement of diaphragm lines)	72	0″.07
Wild Universal theodolite. (Negligible axis strain. Medium suitable arrangement of diaphragm lines)	216	0″.03
Wild Universal theodolite. (Considerable axis strain. Medium suitable arrangement of diaphragm lines)	288	0″.04
Wild Universal theodolite. (Considerable axis strain. Medium suitable arrangement of diaphragm lines. Not cleaned and re-oiled for two years)	72	0″.08

measure, although it is equally certain that in some instruments there exist other unidentified errors which appear to be accidental errors but are not. They are likely connected with the instrumental errors due to axis strain.

Accidental errors may be judged from the "closure" readings on Collimator *A* as shown in Table I. These errors of closure are a measure of the error of the measurement of an angle, and their variation under different instrumental conditions gives an interesting clue to their probable cause. The average value of the closure gives the average error of an angle measured in one "position" due to accidental errors of observation and other unidentified causes. From this average error of closure the average error of the calculated value of the mean diametral strain error has been deduced. Table XI gives these data.

It is believed that Table XI permits the following general conclusions to be drawn:—

1. With Wild Precision theodolites largely free from axis strain, and optical adjustments carefully made, the instrument being cleaned and re-oiled before being used, and with the most suitable diaphragm for making accurate pointings, the accidental errors included in the average diametral strain errors shown in Table III should be no greater than about $0''.01$. These accidental errors were thus smaller than the strain errors which were being measured. *Note*—With this theodolite two micrometer readings are made with each telescope pointing.

2. With the Wild Universal theodolite in good condition (three micrometer readings being taken with each telescope pointing), accidental errors included in the average diametral strain errors shown in Table VIII should be no greater than $0''.03$, of about the same magnitude as the lowest indicated strain errors. That this figure is larger than a corresponding figure with the Precision theodolite may be partly due to the use of a less suitable diaphragm, and perhaps also to a lower precision in the reading of the circle.

In most of the Wild Precision theodolites the telescope was fitted with a glass diaphragm having two short, nearly vertical converging lines ruled thereon; at one end the lines were 20 sec. apart and at the other 40 sec. In one Wild Precision theodolite and all the Wild Universal theodolites the telescopes were fitted with glass diaphragms having two parallel lines ruled thereon about 20 sec. apart. The image of the collimator wire was placed midway between the lines.

More precise pointings could be made with the converging-line diaphragm than with the narrow-spaced parallel lines, as that part of the space which gave the least eye strain could be selected. It is believed that this is one reason why the smaller average accidental error is given in Table XI for the Precision than for the Universal theodolites. Another reason is that the micrometer of the Precision model may be read to $0''.2$, whereas that of the Universal model may be read to $1''.0$.

3. Where considerable axis strain exists, what appear to be accidental errors are larger, the extra amount being probably due to erratic manifestations of frictional errors which are associated with axis strain.

4. Where the theodolite axes have not been cleaned and re-oiled for some time (say two years) the accidental errors are also considerably larger. Where this is associated with strain errors, gummy oil undoubtedly accentuates the frictional errors mentioned in Item 3 above.

Collimator Adjustment

Considerable experience with axis strain tests indicates the necessity of meticulous care in, and regular inspection of, the adjustment of collimators to avoid the possibility of introducing systematic errors larger than the strain errors which are being measured.

When a collimator is adjusted for infinite focus the rays from the objective are parallel, and any eccentricity of the instrument being tested makes no difference in the angle subtended at the instrument by two collimators so adjusted. *In actual practice, however, unless meticulous care is taken in the focusing of the collimators, the above-mentioned condition is difficult of attainment.* In an extreme case it was found that, when the instrument was moved eccentrically by successive amounts of 0.1 in., the angle between two collimators changed on a straight line curve by as much as six seconds per 0.1 in. eccentric movement of the theodolite.

The adjustment of collimators to infinite focus is hence one of prime importance in the test for axis strain, as there is a possibility of a small change in centering when the base of the theodolite is rotated between various sets of measurements.

The procedure employed in the adjustment of collimators used in the tests given in this paper may be useful to those desiring to make similar tests. It is not suggested that the method here described is the only one, or even the best, for adjusting collimators. It was developed by a process of trial and

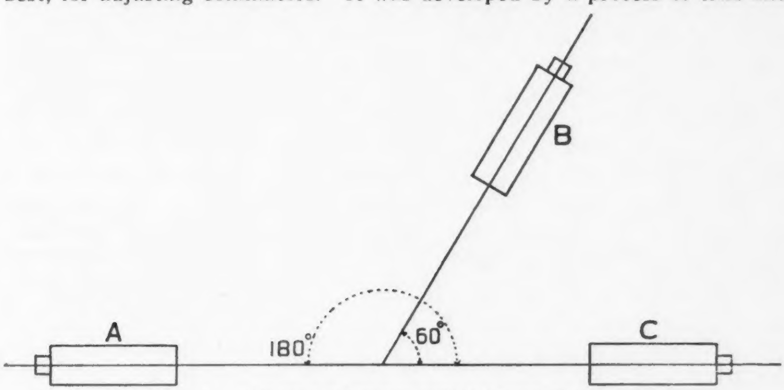

FIG. 5. *Spacing of collimators used in laboratory tests.*

error, and at least has the merit that it yielded satisfactory results. Rather full particulars are given as the authors were unable to find satisfactory references to what is really a very important factor in the accurate measurement of the directions of collimators.

Apart from the two collimators spaced at 60° used in the axis strain test, it was found useful for other purposes to have a third collimator spaced 180° from one of the other two, as shown in the diagram in Fig. 5.

The procedure employed in the adjustment of the collimators is as follows:–

1. The intersection of the cross hairs of the collimators is adjusted to the axis of rotation of the collimators. The method employed is obvious. It may later be necessary to change the cross-wire intersection to bring it to the centre of the illuminated portion of the field.

2. The illumination of the collimator wires is a factor in the sharpness of definition. In the present instance the collimator eyepiece was removed and light admitted through a pin-hole of 1/50 in. diameter, adjustable longitudinally with reference to the collimator axis.

Ground glass and a green filter in front of the light source provided an even illumination of the field which is very easy on the eyes.

Masking the collimator objective to as small an aperture as possible, consistent with clear illumination of the cross wires, minimizes defects of the objective as well as adverse effects of third order aberrations and hence contributes to sharpness of definition.

3. A better target is obtained if the optical axis of the theodolite telescope swings into coincidence with the axes of the collimators. To obtain this coincidence:—

(a) Collimators *A* and *C* are leveled and set as closely as possible to the same height.

(b) These two collimators are exactly lined in by stretching a fine thread between two points exactly over the centres of their eyepieces. A second thread hung vertically between the two collimators in contact with the horizontal thread provides a suitable means of sighting on the collimator objectives for lining them in. The collimators should be moved laterally so that when one is used as a telescope and is pointed on the other as a collimator the thread is exactly over the centres of the apertures of the masked objectives.

(c) The centrally placed theodolite is located so that the axis of its telescope is exactly under the horizontal thread and is at the same elevation as those of the collimators. If the theodolite centre is fixed by circumstances the vertical thread is hung over this centre, and Collimators *A* and *C* are moved laterally until the horizontal thread is in contact with the vertical one.

4. The theodolite on the central pier having been previously adjusted as close to infinite focus as possible by visual methods, Collimators *A* and *C* are visually adjusted to best focus as viewed through the theodolite. With the theodolite removed Collimators *A* and *C* should be closely in focus, one on the other.

5. The theodolite is now turned from Collimator *C* to the required position of Collimator *B*. In setting the latter three conditions should be satisfied:—

(a) Its cross hairs are very nearly 60° from those of Collimator *C*.

(b) Its axis is level and is adjusted to the same height as Collimators *A* and *C*.

(c) By means of threads as described in 3*(b)*, its axis is exactly lined in with the centre of the theodolite.

6. The final focusing of the collimators can now be attempted. For the axis strain test only *B* and *C* are required, and the final focusing is simplified if only *B* and *C* are considered. The procedure used is as follows:—

(a) Focus the theodolite on each collimator repeatedly, marking on the focusing screw of the theodolite the positions of best focus. If the theodolite focus is different on the two collimators, change the focus of one collimator until the theodolite focuses exactly the same on both.

(b) Move the theodolite on the central pier along the bisector of the angle *B* to *C* one-half inch to Position II (Fig. 6) and read the angle *B* to *C*.

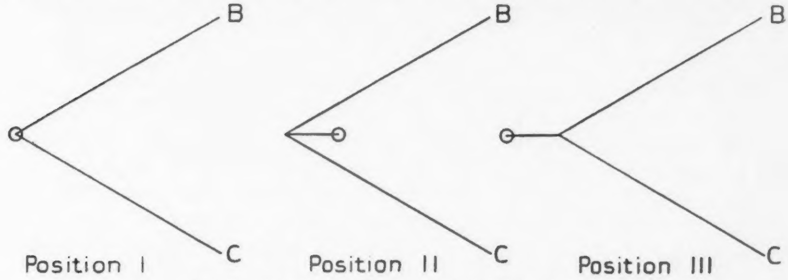

FIG. 6. *Central and eccentric positions of theodolite in test of collimators for infinite focus.*

(c) Move the theodolite along the bisector of the explement of the angle one-half inch to Position III and read the angle *B* to *C*. If the collimators are at infinite focus the angles in Positions II and III should be the same. In practice they usually are different. The mean of the angles in Positions II and III may be called the "true value". It will be very close to that obtained in Position I.

(d) If, in Position II, the measured value of the angle is too large, the condition may be visualized as in Fig. 7; the rays from the collimators are divergent instead of parallel. The focus of both collimators *B* and *C* must be corrected *by the same amount* by trial and error, until the "true value" of the angle is read at Position II. Each change of focus of the collimators requires a change of focus of the theodolite.

(e) If in Position II the measured value of the angle is too small, the rays from the collimators are convergent, and the opposite procedure to that in Item *(d)* is called for.

(f) If the angle in Position III is to be corrected, all effects are the reverse of those in Position II.

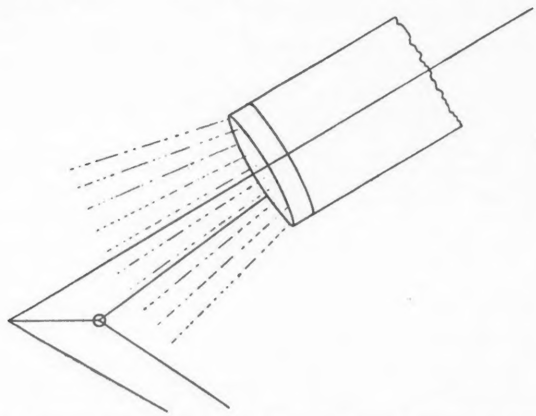

FIG. 7. *Slightly diverging rays from collimators increase the angle measured in Position II of Fig. 6.*

(g) Until the stability of focus of the collimators is established, this test should be made at regular intervals. For this reason it will be found convenient to keep a record of the direction and amount of turning required in the focusing screws of the collimators to produce the desired effect.

(h) During the axis strain tests described in the above paper an effort has been made to keep the angles in Positions II and III within about one second of one another, as there was a maximum change in centering the theodolites of about 1/50 to 1/32 in. with the apparatus used for rotating the bases.

Acknowledgments

The heavy program of observations necessitated by this investigation was performed by three officers of the Geodetic Survey of Canada, Department of the Interior—E. M. Medlen, D. J. Fraser and F. P. Steers. To these and other officers of the Geodetic Survey of Canada, and to R. H. Field, of the Division of Physics and Engineering of the National Research Council, Ottawa, who was frequently consulted and gave valuable advice, the authors gratefully acknowledge their indebtedness.

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CHEESE-RIPENING STUDIES[1]

THE INFLUENCE OF DIFFERENT EXTRACTS ON THE ACID PRODUCTION OF LACTIC ACID BACTERIA

BY WILFRID SADLER[2], BLYTHE ALFRED EAGLES[3], JOHN FRANCIS BOWEN[4] AND ALEXANDER JAMES WOOD[4]

Abstract

The influence of different extracts on the acid production of two strains of *Streptococcus cremoris* and two of *Betacoccus cremoris* isolated from Kingston cheese has been studied.

The enriching entity has no effect on acid production by *Streptococci* in sugar broth. Enrichment with yeast or alfalfa extract causes not only a marked increase in the acid production by *Betacocci*, but also a definite stimulating effect on the rate of acid production.

Enriching milk with yeast or alfalfa extract has a marked stimulating effect on the vital activity of the *Betacocci* and of *Streptococcus* EMB_1173, but is without influence on *Streptococcus* EMB_1195. The response of culture EMB_1173 is immediate and direct, but in the case of cultures EMB_2168 and EMB_2173 the action of the enriching entity is cumulative in its effect. Alfalfa extract would appear to provide, in the case of *Betacoccus* EMB_2173, a stimulating influence not to be found in yeast extract.

The influence of other forage crop enrichments on rate of acid production has also been studied. The factor or factors present in alfalfa and shown to exert a stimulating influence on the vital activity of the organisms are to be found to some extent in all forage crops investigated.

In previous papers (1, 3, 4, 5) the senior writers reported on the influence of the nitrogen source on the sugar-fermenting abilities of the lactic acid bacteria, and showed that the "kind" and "amount" of nitrogen are both critical, and that when yeast extract is used as an enriching entity, specific lactic acid strains demand a certain part of a *particular* nitrogen fraction for the fermentation of a *particular* carbohydrate.

During the study of work in connection with the effect of alfalfa on the nutrition of higher animals (2), it was conceived to be possible that alfalfa might possess some specific stimulating properties, such as, for instance, that exhibited by yeast in the fermentation of sugars by lactic acid bacteria. As the study of the sugar-fermenting abilities of the bacteria progressed, the authors were consequently led to enquire into the effect of alfalfa extract as an enriching medium.

[1] *Manuscript received March 25, 1936.*

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Section I. The Influence of Alfalfa Extract on the Sugar-fermenting Abilities of Lactic Acid Bacteria

For the determination of the comparative stimulatory properties of yeast and alfalfa, mannose was employed as the carbohydrate source. Peptic casein digest broth having a total nitrogen content of 1%, source No. 1, and the same broth diluted to contain approximately 0.5% of total nitrogen, source No. 2, were used as nitrogen providers. Each of the two nitrogen sources was enriched with Bacto yeast extract and with a water extract of alfalfa* respectively—the enrichment in each case being equivalent in terms of nitrogen to an addition of 0.01%† of total nitrogen. All media were brought to equal, and hence comparable, volumes by the addition of the required quantity of water.

The method of preparing the sugar broth and the procedures followed throughout the fermentation study have been described previously (4).

The results of the determinations of total titratable acidity—recorded as grams of lactic acid per litre—produced by cultures EMB_1195 and EMB_2173 (see Section II for classification of organisms) are given in Table I.

TABLE I

Titratable acidity in grams lactic acid per litre (mannose as carbohydrate source)

Culture number	Nitrogen sources**					
	2	2*a*	2*e*	1	1*a*	1*e*
EMB_1195	4.7	4.7	4.7	7.9	7.9	8.1
EMB_2173	5.6	7.4	10.4	11.5	11.7	14.2

** *Nitrogen source No. 2—peptic caseinogen digest broth containing approximately 0.5% of total nitrogen. (Eagles and Sadler, 1932.)*

Nitrogen source No. 1—peptic caseinogen digest broth containing approximately 1.0% of total nitrogen. (Eagles and Sadler, 1932.)

Nitrogen sources Nos. 2a, 1a—Nitrogen sources Nos. 2 and 1 respectively, enriched with Bacto yeast extract at the rate of 0.15%—equivalent to an enrichment of 0.01% of total nitrogen.

Nitrogen sources Nos. 2e, 1e—Nitrogen sources Nos. 2 and 1 respectively, enriched with a water extract of alfalfa—equivalent in terms of nitrogen to an enrichment of 0.01% of total nitrogen.

Whilst the enriching of source No. 2 with yeast extract or with alfalfa extract has no effect on the total titratable acidity produced by culture EMB_1195, it is seen that in the case of culture EMB_2173 there is a marked increase and difference in acid production. When yeast extract is employed as the enrichment there is an increase of 50% in total titratable acidity, but when alfalfa extract is added the increase is approximately 100% (Table I).

When source No. 1 is employed, the total titratable acidity produced by each of the organisms is much greater than the amount produced when source No. 2 is used (Table I)—and previous results are confirmed. The enrichment

* *The authors are indebted to Dr. G. G. Moe, of the Department of Agronomy, for his assistance in the procuring of the alfalfa. The alfalfa used was third generation material from a cross between Medicago media and Medicago falcata.*

† *The water extract of alfalfa contained approximately 0.10% total nitrogen.*

of source No. 1 with yeast or alfalfa extract has no influence on the amount of acid produced by culture EMB_1195. While enrichment of source No. 1 with yeast extract has no effect on the amount of acid produced by culture EMB_2173, there is a definite increase of acid produced by this organism when the broth is enriched with a water extract of alfalfa (Table I).

Considering the results as a whole, the main observation to be made regarding culture EMB_2173 is that a water extract of alfalfa has a stimulating effect greater than that obtained when the broth is enriched with yeast extract.

Section II. The Enrichment of Milk with Yeast Extract and with Alfalfa Extract

In view of the results obtained with alfalfa extract as the enriching entity for casein digest broth, it was thought that the same extract might provide for a marked stimulation of the vital activity of the organisms when used as an enrichment for milk.

For this study the mixed milk of a number of cows was used. In each set of determinations a different milk was employed. Milk from the same herd was used in series numbers 1, 2, 3, 6, 8, 9, 10 and 11, and milk from three separate herds was employed in series numbers 4, 5 and 7 respectively.

In the comparative tests the milk was enriched with Bacto yeast extract as well as with alfalfa extract—the enrichment in each case being the equivalent in terms of nitrogen content of 0.01% of total nitrogen.*

Determinations were made on four cultures isolated from Kingston cheese. The cultural characteristics of the organisms have been described in a previous paper (4). On the sum of their characteristics, cultures EMB_1173 and EMB_1195 (referred to in Section I) are to be classified as strains of *Streptococcus cremoris* (Orla-Jensen). It is to be noted that culture EMB_1173 is atypical in that it responds markedly to the enrichment of the medium with yeast extract. Cultures EMB_2168 and EMB_2173 (referred to in Section I) are to be classified as strains of *Betacoccus cremoris* (Knudsen and Sørensen).

Procedures followed throughout the fermentation study have been described previously (4). The results of the determinations of the total titratable acidity produced by each of the four cultures are given in Table II.

When milk is enriched with yeast extract the total titratable acidity produced by culture EMB_1173 is approximately 300% higher than the amount of acid produced from milk without enrichment, and alfalfa extract is shown to be quite as effective as yeast extract (Table II).

Enrichment of milk with yeast extract or with alfalfa extract has brought about no change in the vital activity of culture EMB_1195.

From Table II it is seen that the enriching of milk with yeast extract or with alfalfa extract has exercised a marked stimulating effect on the amount of titratable acidity produced by cultures EMB_2168 and EMB_2173; and it is

* *The enriched milk was prepared as follows: Mixed Milk a—the necessary weight of yeast extract for 1000 cc. of milk is dissolved in a minimum of water, filtered and made up to 100 cc. volume; Mixed Milk e—100 cc. of alfalfa extract (0.1% of total nitrogen) added to 1000 cc. of milk.*

TABLE II

TITRATABLE ACIDITY IN GRAMS LACTIC ACID PER LITRE

Culture number	Milk* employed as medium	Series number										
		1	2	3	4	5	6	7	8	9	10	11
EMB_1173	Mixed milk	1.8	3.8	1.4	1.4	1.1	1.4	1.4	1.1	0.7	1.4	1.8
	Mixed Milk *a*	4.5	5.2	5.0	5.0	5.9	5.4	6.3	5.9	4.5	5.6	5.0
	Mixed Milk *e*	4.1	5.0	5.0	4.5	5.4	5.4	5.4	5.4	4.3	6.3	5.0
EMB_1195	Mixed milk	6.3	6.8	6.5	6.3	6.8	7.0	7.0	7.0	6.8	6.8	5.6
	Mixed Milk *a*	5.6	6.1	5.9	5.9	6.3	6.8	6.8	7.2	5.9	6.5	6.3
	Mixed Milk *e*	6.1	6.3	7.2	6.1	7.0	6.8	7.0	7.3	6.5	6.5	6.1
EMB_2168	Mixed milk	1.1	0.7	0.7	0.7	0.8	0.7	0.7	1.1	0.7	0.7	1.1
	Mixed Milk *a*	6.5	4.1	4.3	6.8	4.7	6.3	7.9	1.8	4.7	4.1	5.6
	Mixed Milk *e*	7.7	7.2	7.0	5.9	5.9	7.4	7.4	4.7	7.7	6.5	5.9
EMB_2173	Mixed milk	1.8	1.4	1.1	2.0	1.6	1.4	2.3	1.8	1.8	1.6	1.8
	Mixed Milk *a*	3.4	3.6	4.3	5.4	4.3	4.7	7.4	3.8	4.1	3.2	6.1
	Mixed Milk *e*	7.9	8.6	9.0	7.9	7.7	8.1	9.0	8.1	8.3	8.3	7.7

* *Mixed milk used throughout—without additions and with enrichments as defined. For each series a different milk was employed.*

Mixed milk—no additions.

Mixed Milk a—mixed milk enriched with Bacto yeast extract at the rate of 0.15%—equivalent to an enrichment of 0.01% of total nitrogen.

Mixed Milk e—mixed milk enriched with a water extract of alfalfa—equivalent in terms of nitrogen to an enrichment of 0.01% of total nitrogen.

apparent that the influence of the enrichment is more marked in the case of alfalfa extract than in the case of yeast extract.

While it has been stated and shown that for culture EMB_1173 the stimulating effect of alfalfa extract in milk is strictly comparable with the effect of yeast extract, this relation is not observed when the acid production of cultures EMB_2173 and EMB_2168 is considered. In the case of culture EMB_2168 there is an increase of approximately 28% (average figure) in the amount of acid produced when alfalfa extract is employed as the enriching entity, and in the case of culture EMB_2173 there is an increase of approximately 80% (average figure). It would appear, therefore, that for culture EMB_2173 the enriching of milk with a water extract of alfalfa provides for a specific stimulating agency not to be found in yeast extract (Table II).

Section III. The Influence of Yeast Extract and of Alfalfa Extract on the Rate of Acid Production

In Sections I and II of this paper it has been shown that when the cultures are titrated after the standard 14 days' incubation, the enrichment of casein digest broth or of milk with yeast extract or with a water extract of alfalfa causes a marked increase in the amount of total titratable acidity produced by certain of the strains in question. As the work progressed the authors were led to study the effect of yeast and of alfalfa extracts not only on *total acid production*, but also on the *rate of acid production* during succeeding stages of incubation.

For the study of the rate of acid production in sugar broths and in milk,* seven tubes of each medium were uniformly inoculated with the specific culture. After inoculation each series, with controls, was incubated at the appropriate temperature—23° C. At defined intervals the cultures and corresponding control tubes were removed from the incubator, and the amount of titratable acidity was determined.

The results thus obtained for the rate of acid production from glucose and from lactose in broth are tabulated in Table III; and in Figs 1, 2, 3 and 4

TABLE III

RATE OF PRODUCTION OF TITRATABLE ACIDITY

Grams lactic acid per litre

Culture number	Nitrogen source*	Glucose							Lactose						
		Time of incubation in days							Time of incubation in days						
		1	2	3	6	9	12	14	1	2	3	6	9	12	14
EMB_1173	2	2.3	3.8	3.8	4.1	4.3	4.3	4.3	2.5	3.8	3.8	4.3	4.3	4.1	4.1
	2a	3.6	3.8	4.1	4.3	4.7	4.7	4.7	3.4	4.3	4.3	4.3	4.3	4.5	4.3
	2e	3.2	4.3	4.3	4.5	5.0	5.0	5.0	2.7	4.1	4.1	4.3	4.3	4.3	4.3
	1	1.4	6.1	6.3	6.5	7.4	7.7	7.9	3.2	6.8	6.8	7.2	7.7	7.4	7.4
EMB_1195	2	3.6	3.8	3.8	4.1	4.3	4.5	4.5	3.6	4.1	4.1	4.3	4.3	4.3	4.3
	2a	3.6	3.8	3.8	4.3	4.3	4.5	4.5	3.4	4.1	4.1	4.3	4.3	4.3	4.3
	2e	3.6	4.1	4.1	4.3	4.7	4.7	4.7	3.4	4.1	4.1	4.3	4.3	4.3	4.3
	1	5.0	6.3	6.8	7.2	7.9	8.3	8.3	2.3	7.0	7.0	7.4	7.7	7.7	7.7
EMB_2168	2	0.0	0.0	0.5	1.8	2.5	3.2	3.2	0.0	0.2	0.2	1.6	2.5	2.9	2.9
	2a	0.0	1.1	1.8	2.9	3.6	4.1	4.5	0.0	1.6	2.5	3.4	3.6	4.1	4.3
	2e	0.0	2.3	3.4	4.7	5.6	6.5	6.5	0.0	3.8	4.3	5.4	5.9	6.5	7.0
	1	0.0	0.0	0.0	1.8	3.8	5.0	5.4	0.0	0.2	0.2	2.3	3.6	4.7	5.0
EMB_2173	2	0.0	0.0	0.5	1.8	3.7	3.2	3.4	0.0	0.0	0.2	1.4	2.0	2.5	2.7
	2a	0.0	1.1	1.6	2.7	3.4	3.6	4.1	0.0	1.6	2.5	3.4	3.6	4.1	3.8
	2e	0.5	2.3	3.4	4.1	5.2	5.6	6.1	0.7	3.8	4.5	5.4	5.9	6.3	7.2
	1	0.0	0.0	0.2	2.7	4.7	5.0	5.9	0.0	0.2	0.5	2.0	2.7	4.3	5.0

* *Nitrogen source No. 2—peptic caseinogen digest broth containing approximately 0.5% total nitrogen. (Eagles and Sadler, 1932.)*

Nitrogen source No. 2a—Nitrogen source No. 2 enriched with Bacto yeast extract at the rate of 0.15%—equivalent to an enrichment of 0.01% of total nitrogen.

Nitrogen source No. 2e—Nitrogen source No. 2 enriched with a water extract of alfalfa—equivalent in terms of nitrogen to an enrichment of 0.01% of total nitrogen.

Nitrogen source No. 1—peptic caseinogen digest broth containing approximately 1.0% of total nitrogen. (Eagles and Sadler, 1932.)

the results for lactose are expressed graphically. The results of the determination of the total titratable acidity produced at defined times throughout the 14 days' incubation period in milk alone, in milk enriched with yeast extract, and in milk enriched with alfalfa extract, are presented graphically in Figs. 5, 6, 7 and 8.

* *Mixed milk was used throughout. With essentially the same results the work has been repeated on five separate occasions, using for each set of determinations a different milk.*

The total titratable acidity produced by cultures EMB_1173 and EMB_1195 from glucose and lactose is about 100% higher when the nitrogen source is peptic casein digest broth containing approximately 1% of total nitrogen, source No. 1, rather than the same broth diluted to contain approximately 0.5% of total nitrogen, source No. 2. When source No. 2 is employed the total titratable acidity produced by each of these organisms is closely comparable with that produced when the nitrogen is supplied as source No. 2 enriched with yeast extract or with alfalfa extract (Table III; Figs. 1 and 2).

For culture EMB_1173 the curves for the rate of acid production in nitrogen sources Nos. 2, *2a* and *2e* are practically identical. At no stage of the incubation period does the enriching entity appear to exert a stimulating effect. Culture EMB_1173 has produced its maximum titratable acidity within 48 hr. It is to be noted that the curves for rate of acid production in source No. 1 and in source No. 2 are comparable, the production of acid in source No. 1 proceeding at the same rate as in source No. 2, but at a higher level.

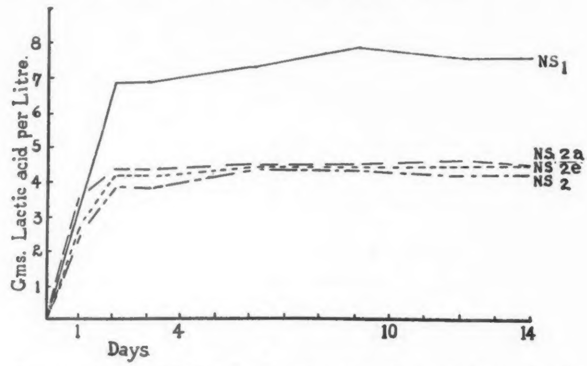

FIG. 1. *Culture EMB_1173. Rate of acid production in lactose broth.*

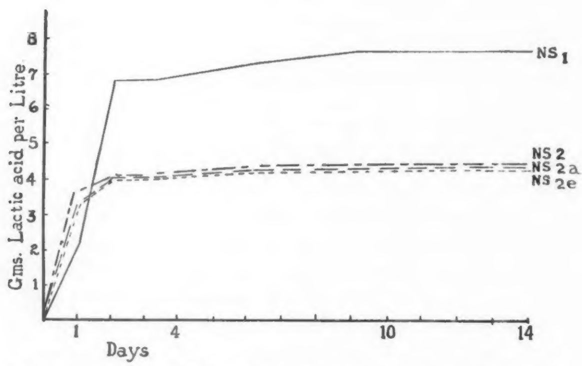

FIG. 2. *Culture EMB_1195. Rate of acid production in lactose broth.*

In the case of culture EMB_1195, the curves for rate of acid production in each of the nitrogen sources are practically identical with the corresponding curves for culture EMB_1173.

The total titratable acidity produced by cultures EMB_2168 and EMB_2173 from glucose and lactose in source No. 1 is much higher than is the case when source No. 2 is employed. It is to be seen that the total acidity produced by these organisms in sources Nos. 1 and 2, respectively, is much lower than that produced by cultures EMB_1173 and EMB_1195 in the same nitrogen sources. When source No. 2 is enriched with yeast extract, the total titratable acidity produced is approximately equal to that produced with source No. 1. The total titratable acidity produced by these cultures is still greater, however, when the basic broth, source No. 2, is enriched with alfalfa extract, and is comparable with the total titratable acidity produced by cultures EMB_1173 and EMB_1195 in source No. 1 (Table III; Figs. 1–4).

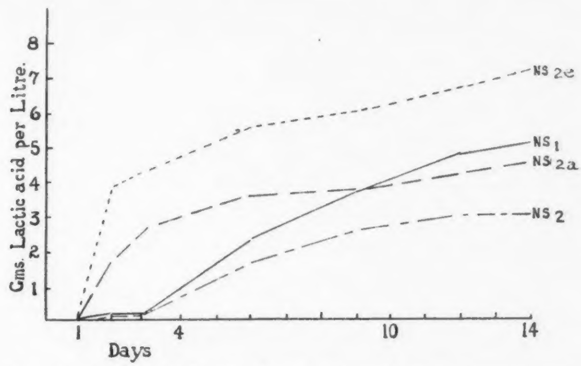

FIG. 3. *Culture EMB_2168. Rate of acid production in lactose broth.*

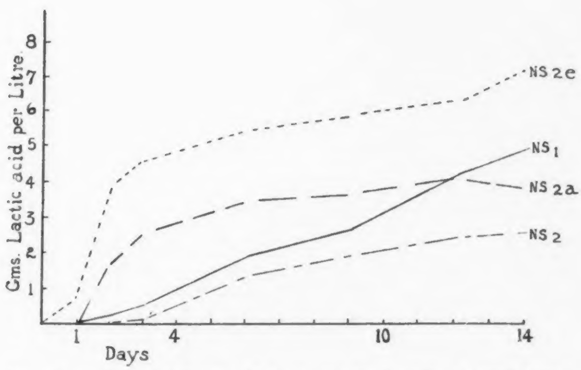

FIG. 4. *Culture EMB_2173. Rate of acid production in lactose broth.*

For each of the organisms, cultures EMB_2168 and EMB_2173, the curves for rate of acid production in sources Nos. 1 and 2 are comparable the one with the other, as was shown in the case of cultures EMB_1173 and EMB_1195.

A critical difference in the rate of acid production is to be seen, however, when the curves for cultures EMB_2168 and EMB_2173 in sources Nos. 1 and 2 are compared with those for cultures EMB_1173 and EMB_1195 in the same nitrogen sources. Whilst the maximum acidity produced by cultures EMB_1173 and EMB_1195 is reached at the end of 48 hr. incubation, this point is not reached in the case of cultures EMB_2168 and EMB_2173 until near the end of the 14-day period.

For these cultures, when source No. 2 is enriched with yeast extract or with alfalfa extract, one may observe not only a marked increase in total acid production, but also a definite stimulating effect on the rate of acid production (Table III; Figs. 3–4).

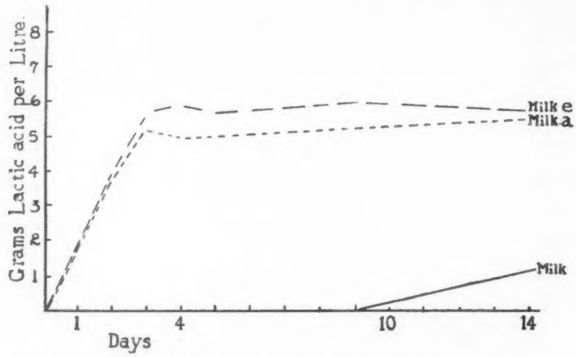

FIG. 5. *Culture EMB_1173. Rate of acid production in milk.*

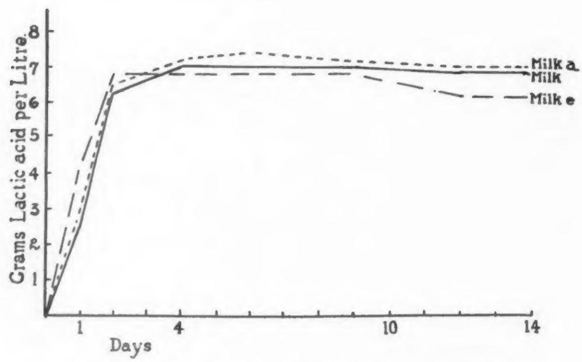

FIG. 6. *Culture EMB_1195. Rate of acid production in milk.*

The response of cultures EMB_2168 and EMB_2173 to the enrichment of source No. 2 with alfalfa extract is immediate and direct, and continues throughout the period of incubation. The effect of yeast extract enrichment in the case of broth would appear to be restricted to a gradual stimulation of activity throughout the whole period. Within 48 hr. after inoculation the total titratable acidity produced from alfalfa-enriched broth is as great as that produced at the end of 14 days from the same broth enriched with yeast extract.

When vital activity is defined in terms of the the total titratable acidity produced after incubation for 14 days, it is seen that the enriching of milk with yeast extract or with a water extract of alfalfa has a marked stimulating effect on the vital activity of cultures EMB_1173, EMB_2168 and EMB_2173, and is without influence on culture EMB_1195 (Figs. 5–8)—confirming previous results. For culture EMB_1195, the curves for the rate of acid production in milk, in milk enriched with yeast extract, and in milk enriched with alfalfa extract, are practically identical. At no stage of the incubation period does

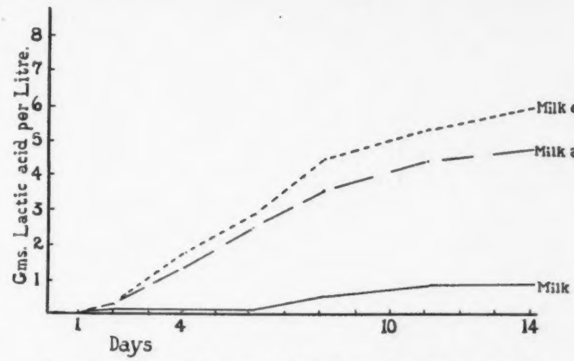

FIG. 7. *Culture EMB_2168. Rate of acid production in milk.*

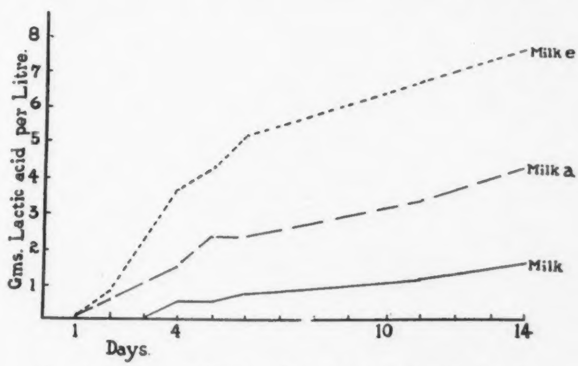

FIG. 8. *Culture EMB_2173. Rate of acid production in milk.*

the enriching entity appear to exert a marked influence on this organism, except for a slight stimulating effect of yeast extract in the first 24 hr. Culture EMB_1195 has produced its maximum titratable acidity within 24 hr.

Cultures EMB_1173, EMB_2168 and EMB_2173 produce a small amount of total titratable acidity after 14 days' incubation when grown in milk without enrichment. When milk is enriched with yeast extract or with alfalfa extract, however, the total titratable acidity produced by these three cultures is comparable with the high total titratable acidity produced by culture EMB_1195 (Figs. 5–8).

Apart from the production of acid when grown in milk without enrichment, culture EMB_1173 is comparable with culture EMB_1195 in its ability to form titratable acid, whether milk enriched with yeast extract or with alfalfa extract is employed (Figs. 5–6).

For culture EMB_1173, the stimulating effect of alfalfa extract on the rate of acid production in milk is comparable to the effect obtained by enrichment with yeast extract. Within 24 hr. of the time of inoculation, definite evidence of the stimulating action is to be seen, and within three days maximum acid production has been reached.

Whilst it has been shown that the response of culture EMB_1173 to enrichment of milk with yeast or alfalfa extract is immediate and direct, it is to be seen that for cultures EMB_2168 and EMB_2173 the stimulating influence of the enriching entity is not apparent until after 48 hr. incubation; and it would appear that, for these organisms, the action of yeast extract or of alfalfa extract on the rate of acid production in milk is cumulative in its effect, stimulating the vital activity of the cultures throughout the entire period of incubation.

The marked influence of a water extract of alfalfa on the vital activity of culture EMB_2173 is to be noted from the increase in the amount of acid produced throughout the period of incubation, when the enriching entities are yeast and alfalfa extracts respectively. It would appear that for culture EMB_2173 the enriching of milk with a water extract of alfalfa provides for a stimulating influence not to be found in yeast extract—and previous results are confirmed.

Section IV. The Influence of Other Forage Crop Extracts on Rate of Acid Production

In the light of the results obtained from a study of the stimulating effect of alfalfa extract when used as an enriching entity for the lactic acid bacteria, it was thought desirable to determine whether or not the other forage crops might exhibit similar properties.

For this study, aqueous extracts of the following common forage crops were prepared by a method similar to that used in the preparation of alfalfa extract:

1. *Medicago media*—Alfalfa
2. *Trifolium pratense*—Common red clover
3. *Dactylis glomerata*—Orchard grass

4. *Phleum pratense*—Timothy
5. *Melilotus alba* and *M. flava*—Sweet clover
6. *Avena elatior*—Tall oat grass
7. *Holcus lanatus*—Yorkshire fog
8. Mixed hay (containing perennial rye grass and clover).

The results of determinations on the rate of acid production by culture EMB_2173 when grown in milk enriched with extracts from the plants cited above are expressed graphically in Fig. 9.

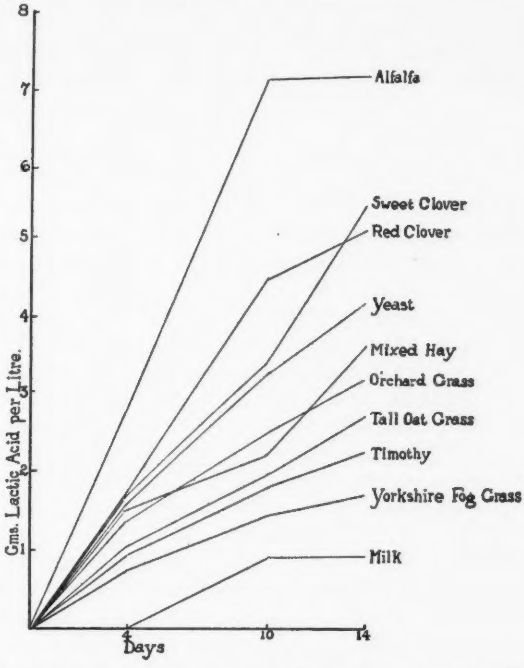

FIG. 9. *Culture EMB_2173. Rate of acid production in milk, and in milk enriched with crop extracts.*

Considering the results as a whole, it is apparent that the factor or factors present in alfalfa, and shown to exert a stimulating influence on the vital activity of culture EMB_2173, are to be found to some extent in all forage crops studied.

Alfalfa, however, would appear to possess some stimulating powers not to be found in other forage crops. In a lesser measure, enriching extracts prepared from red clover and from sweet clover have exerted a marked stimulating effect on the rate of acid production. The total titratable acidity produced by culture EMB_2173 in milk enriched with yeast extract is definitely less than that produced in milk enriched with an extract of alfalfa, red clover or sweet clover respectively.

When the influence on the rate of acid production exhibited by alfalfa extract is compared with that brought about by extracts of the various grasses, it seems probable either *(a)* that the stimulating factor in the grass extracts is not as potent or concentrated as it is in alfalfa extract; or, *(b)* that we are confronted with the effects of more than one stimulating factor, and that certain of these factors present in alfalfa are non-existent in the grasses.

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CHEESE-RIPENING STUDIES[1]

Wildiers' Bios and the Lactic Acid Bacteria

THE FRACTIONATION OF BIOS FROM ALFALFA AND THE EFFECT OF THE FRACTIONS OBTAINED ON THE VITAL ACTIVITY OF THE *BETACOCCI*

By Blythe Alfred Eagles[2], Alexander James Wood[3] and John Francis Bowen[4]

Abstract

The influence of the Bios of Wildiers on the vital activity of two strains of *Betacoccus cremoris* has been studied. Alfalfa, yeast and tomatoes have been fractionated after the manner of Miller, and the effect of the respective Bios fractions on acid production determined. It has been shown that the *Betacocci* demand, for their most intensive metabolism, activators corresponding to those required by yeasts—Bios I, II *A* and II *B*.

In a previous paper (4) the authors showed that extracts of alfalfa and other forage crops, when used as an enriching entity, exert on the acid production by *Betacocci* and certain *Streptococci* a stimulating influence similar to that of yeast extract. It was also shown that, for the culture EMB_2173, alfalfa extract provides a stimulating influence not to be found in yeast extract.

As the work progressed the authors were led to enquire into the nature of the factor or factors concerned. A review of the literature on the question of accessory growth stimulants suggested that a relation might exist between the unknown entities, which the writers had found to exert a marked influence on the acid-producing abilities of the lactic acid bacteria, and the factors essential for the growth of yeasts—the Bios of Wildiers.

Applying the technique developed by Narayanan (2) for the isolation of bios from yeast, the fractionation of alfalfa meal* was undertaken, and the various fractions obtained were added to milk as enrichments. The effect of the added material on the acid-producing ability of cultures EMB_2168 and EMB_2173 was then determined. Results obtained in these determinations showed that the relative strength of the fractions diminished, rather than increased, as the fractionation of the extract proceeded.

The work of Narayanan (2) indicates that the Bios of Wildiers for the growth of yeasts is a single entity. The authors' early results would suggest that, at least for the *Betacocci*, the stimulating effect is due to the influence of *more than one* factor. In later research it appeared probable that the factors with which the writers were confronted might be identical with those reported upon by Miller, Eastcott and Maconachie (1). These investigators have demonstrated a multiple nature of Wildiers' Bios for the growth of yeasts, and have also suggested a wide distribution of these factors in nature.

[1] *Manuscript received March 25, 1936.*
Contribution from the Department of Dairying, The University of British Columbia, Vancouver, Canada.
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[3] *Carnegie scholar, Department of Dairying, The University of British Columbia.*
[4] *Graduate student, Department of Dairying, The University of British Columbia.*
* *The meal used was a dehydrated product prepared from alfalfa leaves and blossoms in such a manner as to preserve the vitamin content of the original material.*

In an attempt to establish the validity of the writers' assumption, fractionation of alfalfa meal was undertaken after the manner of Miller *et al.* (1) for the isolation of the Bios I, Bios II *A* and Bios II *B* from yeast.

The alfalfa bios fractions thus obtained were added singly and in combination as enriching entities to milk, and the effect of the fractions on the acid-producing abilities of cultures EMB_2168 and EMB_2173 were determined. Procedures followed throughout the fermentation study have been described in previous papers (4, 5). The results of the determinations are expressed graphically in Figs. 1 and 2.

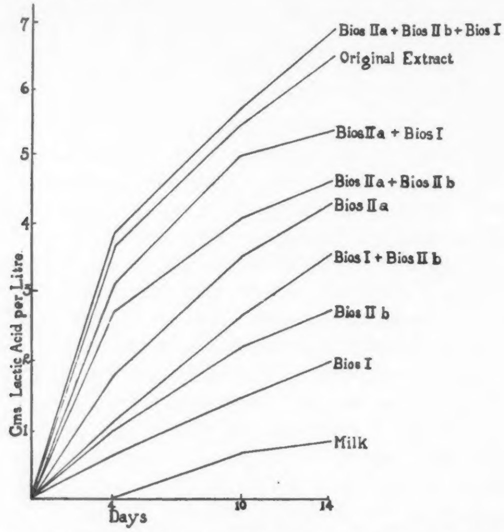

FIG. 1. *Culture EMB_2173. Rate of acid production in milk, and in milk enriched with Bios fractions.*

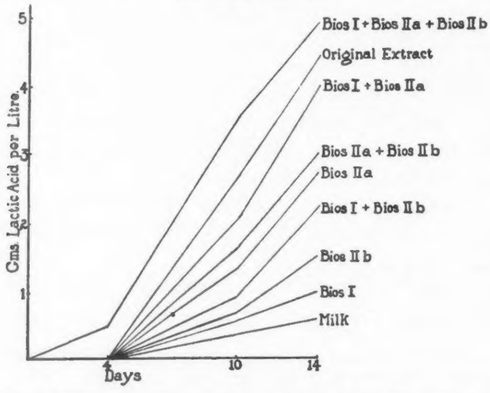

FIG. 2. *Culture EMB_2168. Rate of acid production in milk, and in milk enriched with Bios fractions.*

Through the courtesy of Prof. W. Lash Miller, the authors were supplied with Bios II *A* and Bios II *B* fractions prepared from tomatoes in the Toronto laboratories. When these fractions were used as enriching entities, results similar to those detailed in Figs. 1 and 2 for the alfalfa fractions were obtained. Tomato-bios fractions prepared in the writers' laboratory gave similar results.

When the data for rate of acid production in milk and in milk enriched with Bios I (Inositol), Bios II *A* and Bios II *B* are considered, it is obvious that our hypothesis relative to the nature of the factors present in alfalfa, and shown to be responsible for the increased vital activity of the *Betacocci*, becomes tenable. When used as a single enriching entity, each of the bios fractions exerts a definite effect on acid production. The specific quantitative requirements of the *Betacocci* for the respective bios fractions have not as yet been determined, but it would appear that the effect of Bios II *A* on the rate of acid production is more marked than that of either of the other bios fractions.

When the effects of the enriching entities added in combination are observed, it becomes apparent at once that the influence of alfalfa extract on the acid-producing abilities of the *Betacocci* is due to the activity of more than one stimulating factor. The addition of a second bios to milk containing one of the factors has a marked stimulating effect on vital activity, and when the third bios is added, perfecting the bios complex, the total titratable acidity equals or exceeds that produced by the original water extract from which the fractions were prepared. Considering the results as a whole, it is evident that certain lactic acid bacteria demand, for their most intensive metabolism, activators corresponding to those required by yeasts—Bios I, Bios II *A* and Bios II *B*.

The results of the writers' work on the activator requirements of certain *Betacocci* and *Streptococci* take on a new significance when considered in the light of the findings recently reported upon by Orla-Jensen, Otte and Snog-Kjaer (3). They have shown that for the growth of the "lactic acid bacteria preferring milk as a nutrient medium", 'milk bios' and lactoflavin are essential. Removal of these constituents by adsorption with charcoal renders milk unsuitable for the growth of these organisms. The present authors' results demonstrate that, for the *Betacocci* and for such *Streptococci* which normally find milk a poor nutrient substrate, the enrichment of milk with activators (Bios I, II *A* and II *B*) prepared from alfalfa, yeast or tomatoes, assures ideal growth conditions.

Orla-Jensen *et al.* (3) have postulated the existence of a factor other than 'milk bios' and lactoflavin present in milk and essential for the growth of certain lactic acid rod forms. It would appear from the writers' work that, if the activator requirements of the *Betacocci* are indentical with the requirements of other lactic acid bacteria, the amounts of these substances present in average milk are inadequate for their needs, or that the *Betacocci* demand

activating factors not to be found in all milk, but present in alfalfa, yeast and tomatoes and shown to be identical with Bios I, Bios II *A* and Bios II *B* of Miller *et al.* (1).

Work on the activator requirements of the *Betacocci* and other true lactic acid bacteria is proceeding in this department, while another department of the university is pursuing studies on the effect of corresponding stimulants on the nodule bacteria.

Acknowledgment

The authors wish to express their thanks to Prof. P. A. Boving for much helpful criticism in the preparation of the manuscript.

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THE LIPID COMPOSITION OF THE GUINEA PIG PLACENTA[1]

By Eldon M. Boyd[2]

Abstract

The lipid composition of the guinea pig placenta was found to vary with the duration of pregnancy. Between the 20th and the 40th days there occurred an increase in phospholipid and free cholesterol, both of which remained elevated from then on to term. There was no significant change at any time in the amount of cholesterol esters, but that of neutral fat increased steadily sixfold and more during pregnancy. These changes were interpreted as signifying a gradual change in placental lipid metabolism during pregnancy. The relation of this change to the transfer of lipids from mother to fetus, and its relation to the etiology of the lipemia of pregnancy in guinea pigs, are discussed.

Introduction

It has been shown by Boyd (2) that, as pregnancy advances in rabbits, changes occur in the lipid composition of the placenta. These changes were interpreted as signifying a decrease in the physiological activity of the placental tissues up to about the mid point of gestation, followed by an increased function in the latter half. Correlating this with variations in the lipid composition of the rabbit fetus, it was postulated that the placental tissues of the rabbit take an active part in transferring lipids from the mother to the offspring *in utero*, especially in the latter part of pregnancy.

The placenta of the guinea pig (*Cavia porcellus*) has been selected for a similar study in the present communication. The guinea pig was chosen because pregnancy in this species bears many points of resemblance to human gestation. Pregnancy lasts longer in the case of guinea pigs than it does in other laboratory animals; the young are well developed at birth and capable of living independently of the mother; in gross and microscopic appearance the placenta is like that of women; there is a lipemia during pregnancy in guinea pigs and in women, but not in most other species studied to date (4). As far as the author is aware, no previous studies have been reported on changes in the lipid composition of the placenta in gravid guinea pigs.

Experimental

A colony of about 25 healthy, mature, virgin, female guinea pigs was isolated and each animal examined daily. Oestrus appeared about once every fortnight, and was indicated by the presence of vaginal secretions (from the degenerative desquamation of the uterine and vaginal mucosa), followed by opening of the vagina for a period of some three days. During this interval the female was put into a cage with a healthy buck guinea pig. If oestrus did not reappear in from two to three weeks, the mating was considered to have been fruitful. At various intervals during gestation an hysterotomy was performed on one or more animals, the placenta or placentas removed, weighed,

[1] *Manuscript received February 20, 1936.*
Contribution from the Department of Pharmacology, Queen's University, Kingston, Ontario, Canada.
[2] *Lecturer in Pharmacology, Queen's University.*

ground with sand, extracted, and analyzed for lipids in the same manner as were the placentas of rabbits (2). In all, 18 placentas were so treated, from the 15th day of pregnancy to term at 62 days.

The various lipid fractions determined included total lipid, neutral fat, total fatty acids, phospholipid fatty acids, cholesterol ester fatty acids, neutral fat fatty acids, total cholesterol, ester cholesterol, free cholesterol and phospholipid.

Results and Discussion

Variations in six of these lipids are shown in Figs. 1 and 2. In rabbits (2) it was found that changes which occurred in the first half of gestation were the

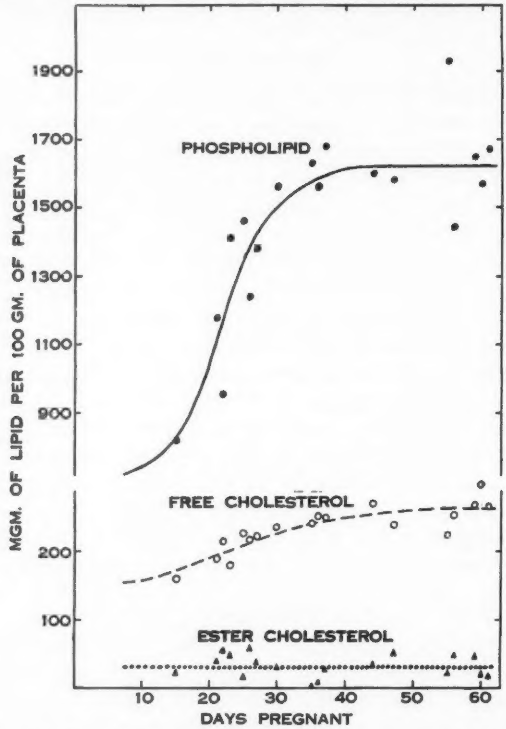

FIG. 1. *Changes in content of placental phospholipid, free cholesterol and ester cholesterol during pregnancy in guinea pigs.*

opposite of those in the second half. In guinea pigs however there was only one general change, an increase in the concentration of certain lipids which began about the 20th day and progressed on to term.

These changes may be seen to have consisted first of all in an increase in placental phospholipid (Fig. 1). The lowest value of 820 mg. per cent was obtained in a placenta taken about the 15th day. The highest value was

1930 mg. per cent from a placenta taken on the 55th day. The phospholipid content doubled as pregnancy advanced, the rate of increase being greatest between the 20th and 30th days. After the 40th day, placental phospholipid content had reached a high plateau level, and there was little further change from then on to term.

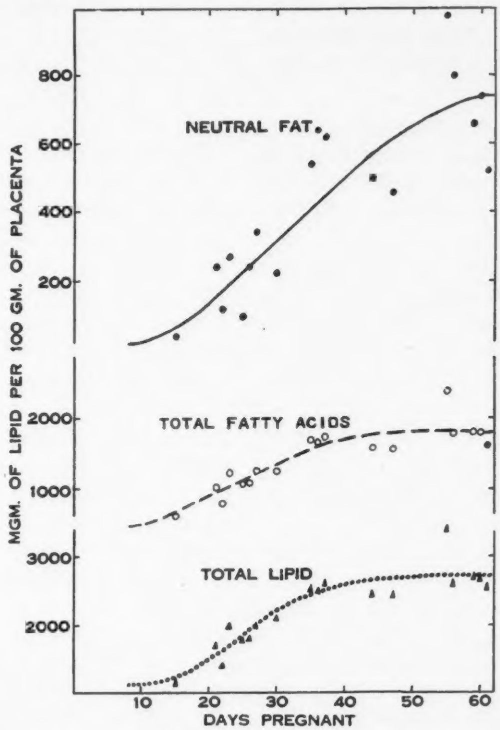

FIG. 2. *Changes in content of placental neutral fat, total fatty acids and total lipid during pregnancy in guinea pigs.*

The increase in placental phospholipid preceded by two weeks or more the increase that occurs in maternal plasma lipids, the amounts of which, as Boyd and Fellows (4) have shown, begin to rise and produce a lipemia after the 40th day. It is possible that the lipemia may be related etiologically to the previous changes in the placenta. Boyd (2) has suggested that the increased phospholipid of the rabbit placenta in late pregnancy may signify an active absorption of lipids from maternal blood by the placenta, and the transference of the same to the fetal circulation. If this be so, then the increased amounts of phospholipid of the guinea pig placenta may bear the same interpretation. And as a result of this increased withdrawal of lipids from the maternal circulation by the placenta, the mother guinea pig responds

by mobilizing lipids into the blood stream and producing a lipemia. As a matter of fact one theory that has been frequently advanced as the cause of the lipemia of pregnancy in man, the so-called "fetal need" theory (1), postulates this very thing without evidence as to how it may be brought about. A fact that is difficult to reconcile with this line of reasoning is that the same stimulus does not produce the same response during pregnancy in rabbits. It is possible that the explanation may lie in referring the blood changes to the stage of development of the fetus and not to the stage of pregnancy.

Free Cholesterol

The free cholesterol content of the placenta also increased during pregnancy. The lowest value was 160 mg. per cent at the 15th day, and the highest was 298 mg. per cent near term. The increase was not as completely limited to the first three or four weeks of gestation as was phospholipid. Free cholesterol rose in value from the beginning to the end, but the rate of increase was somewhat greater in the first than in the second half. This change may refer either to the maintenance of the usual balance between phospholipid and cholesterol in vital economy, or to some especial function played by cholesterol in placental metabolism. The increase in placental free cholesterol content began before the increase in that of plasma free cholesterol, as found by Boyd and Fellows (4).

Ester Cholesterol

There was no significant change in the amount of placental ester cholesterol (Fig. 1). Throughout gestation the placenta contained small amounts of this lipid, between 0 and 54 mg. per cent. Cholesterol esters were found to increase in early pregnancy in rabbit placentas (2), a change which in the light of subsequent work (3) may be interpreted as signifying degeneration rather than inactivity *per se*. The fact that no such change occurred in cholesterol esters in guinea pigs suggests that at no time during early or late pregnancy does the placenta degenerate. And this conclusion is further borne out by the changes in phospholipid and free cholesterol content, both of which vary in the direction of increased physiological activity, as interpreted in terms of Bloor's hypothesis of the relation of lipid composition to physiological activity (see (2) and (3)).

Neutral Fat

Of all the lipids studied, neutral fat (Fig. 2) exhibited relatively the greatest change. Placental neutral fat increased in value from 40 mg. per cent at the 15th day to almost 1000 mg. per cent in a 55 day organ. The rate of increase was fairly regular throughout gestation, perhaps tending to slow up somewhat in the last week. The significance of this change is rather difficult to visualize. Neutral fat is usually regarded as a storage or depot fat, seldom present in large amounts in active cells. However, there has never been found a very consistent relation between the neutral fat content of a tissue and its degree of physiological activity. Boyd and Fellows (4) found that the plasma of gravid guinea pigs contained about 100 mg. per cent of neutral fat up to the

40th day, after which it rose to 300 mg. per cent by the 50th day and to 500 mg. per cent at term. It may be seen in Fig. 2 that placental neutral fat content had increased considerably by the 40th day, before any increase occurred in maternal plasma. It is probable that placental neutral fat may likewise represent some mechanism of transferring lipids from the maternal to the fetal circulation.

As a result of these changes in phospholipid, free cholesterol and neutral fat, it was found, as would be expected, that the total fatty acids and total lipid of the placenta increased as gestation proceeded. These results have been shown in Fig. 2. The total fatty acids increased from about 600 mg. per cent in early pregnancy to about 1800 mg. per cent at term. Total lipid rose from some 1200 mg. per cent to some 2800 mg. per cent at term. In both instances the major increase occurred before the 40th day.

Conclusion

These several changes in lipid composition indicate that placental lipid metabolism undergoes an alteration as pregnancy advances in guinea pigs. The nature of the lipid changes suggests an increase in physiological activity beginning as early as the 20th day. It has been postulated that this increase in physiological activity is concerned with the transfer of lipids from mother to fetus through the mediation of placental tissues. It is equally possible that it may be related to other functions of the placenta, such as the production of sex hormones. Whether these changes are connected with the etiology of the lipemia of pregnancy in guinea pigs is a moot question, but it does appear significant that the lipemia begins to develop some days *after* the changes in the placenta.

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CONTRIBUTIONS TO THE BIOCHEMISTRY OF BROMINE. I.[1]

By A. H. Neufeld[2]

Abstract

A number of recent methods for the estimation of bromine in plant and animal tissues have been tested. A combination of the methods of Francis and Harvey (34) and of Yates (98), with some modifications, has been proved capable of yielding controlled and accurate results for amounts of bromine not less than 3 micrograms, in amounts of material of not more than 1 gm.

Using the method outlined, material from a large number of marine plants and animals and from typical land plants and animals has been examined for bromine content; in many cases iodine analyses were either already available or have been made on the same material. The results suggest the following conclusions:

Bromine is an invariable constituent of marine algae, but no definite relation appears to exist between their bromine and iodine contents. Land plants contain considerably less bromine. Environmental conditions, and perhaps selective affinity by the species and cells in the different parts of the individual plant, probably determine the actual content of bromine.

All the marine species of animals examined contain bromine; but in very variable quantity. Environment and selective cell affinity appear to be the controlling factors.

The thyroid and blood of the mammals completely examined (rat, rabbit, and dog) contain amounts of bromine slightly higher than those present in other tissues. Ox, sheep and hog thyroids contain similar amounts. Pituitary tissue contains amounts scarcely, if at all, greater than most of the other tissues of the mammalian organism; this last finding is not in agreement with statements by certain other investigators.

No relation has been found to exist between the distribution of bromine and iodine in mammalian tissues, and, so far, it is uncertain whether bromine is of functional significance, especially since it does not appear to be particularly associated with the thyroglobulin of the thyroid.

PART I. THE ESTIMATION OF BROMINE IN PLANT AND ANIMAL TISSUES

Introduction

During the last few years a number of new micro-methods for the estimation of bromine in biological material have been described. Results obtained by the use of certain of these suggested that bromine is of somewhat greater metabolic importance than was heretofore thought. Thus it has been concluded that it may be of endocrine significance since it seemed to be particularly associated with the pituitary and the thyroid, while it has been stated that cyclical variations in blood bromine occur during the menstrual cycle (51); there seems also to be some evidence of an abnormal bromine metabolism in certain psychoses (79, 101, 104).

The significance of such conclusions renders it essential that the results on which they are based should depend on sound methods; this has been by no means established.

[1] *Manuscript received April 27, 1936.*
Contribution from the Department of Biochemistry, Faculty of Medicine, University of Manitoba. The work in Part I of this paper fulfilled part of the requirements for the degree of Master of Science at the University of Manitoba.

[2] *Part-time Demonstrator in Biochemistry, University of Manitoba.*

Part I of this paper records the results of a critical examination of certain of the newer methods; a modified combination of two of these appears to give consistent and accurate results. In Part II are outlined systematic analyses, obtained by the use of this procedure, of normal plant and animal tissues, and earlier findings by other investigators are contrasted with these new figures. In later papers results obtained with normal and pathological human material, and other studies, will be reported.

In checking the various analytical procedures, known mixtures of potassium bromide (and other halides), of dibromtyrosine, and of dibromcresol have been employed. These compounds were all of known purity.

Examination of Different Methods

(i) *The Møller Method (65)*

This is designed for fluids. The halogens are converted to silver halides by boiling the material in a mixture of silver nitrate-nitric acid in presence of hydrogen peroxide. The halides are reduced by sodium hyposulphite, and the silver is removed by filtration. (Møller recommends filtration through Allihn's special filter tubes. These were not available, but it is very doubtful whether the more usual technique employed markedly affected the results.) The halide mixture is then oxidized by a permanganate-pyrosulphate mixture, and the bromine aspirated into iodide solution; the liberated iodine is titrated against thiosulphate.

The writer's results obtained by this method were uniformly low, error ranging from 30 to 50%. Lengthening the period of aeration lessened the error, but not sufficiently to render the procedure of value. It seems possible that the permanganate-pyrosulphate mixture is inefficient as an oxidizer.

(ii) *The Leipert and Watzlawek Method (54)*

This also is designed only for fluids. The material for analysis is oxidized by a silver sulphate-chromic acid-sulphuric acid mixture. Iodine is retained as iodate; chlorine and bromine are aspirated into sodium hydroxide solution, and the bromate formed is estimated iodometrically. The authors prescribe a very complicated glass apparatus. An old-fashioned three mouthed Wolff bottle was adapted and found just as suitable.

The method gives good results. In presence of similar amounts of iodine and of excess of chlorine, analyses of amounts of bromine varying from 0.1 to 2 mg. gave errors varying between (maxima) -5.7 and $+6.9\%$, the average error in 15 determinations being 0.5%, and the probable error 2.3%.

The procedure is rather difficult, and is costly, large amounts of reagent being required for each determination.

(iii) *Oxidation Methods*

Various oxidation procedures have been contrasted. The main difficulty in these methods lies in effecting efficient separation of chlorine and bromine.

(a) The Francis and Harvey method (34). A chromic acid-phosphoric acid mixture is used. Bromine is aspirated off into iodide solution, and estimated iodometrically.

The present writer obtained very low results with this method. With amounts of bromine varying from 0.03 to 0.08 mg., errors ranged from −18 to −73%. The oxidation mixture seems inefficient.

(b) Bertram's method (9). The bromide is oxidized to bromine with boiling potassium permanganate, acidified by potassium hydrogen sulphate and buffered with potassium sulphate. Bromine is distilled into 0.1% potassium iodide solution, and this, after acidification, is again distilled and the second distillate titrated against thiosulphate.

In a series of analyses of material containing amounts of bromine ranging from 0.02 to 0.2 mg., the results were invariably low, the error ranging from −16 to −29%. Probably this error is due in part to insufficient oxidation and in part to loss of bromine through the repeated distillation; the iodide solution specified is obviously very dilute.

(c) Yates' method (98). The mixture of halogens is oxidized with a chromic acid-sulphuric acid mixture within a definite pH range. Under specified pH conditions all bromide is oxidized to bromine in the cold, chloride remains unaffected, and iodide is changed to iodate. The bromine is aspirated into 10% potassium iodide and the iodine liberated is titrated against $N/500$ thiosulphate.

The writer found this method very satisfactory with amounts of bromine ranging from 0.009 to 0.9 mg. In eight estimations the maximum errors were −5.3 and +8.8%, the average error −0.4%, and the probable error 2.5%.

Both direct sunlight and darkness must be avoided. The former leads to oxidation of chloride and, in consequence, to results that are too high, while the latter leads to incomplete oxidation of bromide.

(iv) *Ashing Methods*

By means of Yates' oxidation procedure it was possible to investigate the accuracy of ashing methods. Francis and Harvey's method (34), slightly modified, was found to give satisfactory results. Francis and Harvey incinerate the material first in a nickel crucible and finally in a platinum crucible. Various writers claim that incineration of the sample in nickel vessels leads to low results.

The results obtained in the present investigation showed that when the incineration is immediately followed by oxidation, accurate results are obtained with nickel vessels. If the incinerated material is allowed to stand overnight in a nickel crucible, low results follow through loss of bromine.

As a result of the preliminary work just recorded the modified Francis-Harvey incineration, combined with the Yates' oxidation technique, has been adopted as a standard procedure and thoroughly tested. It is outlined below.

The Modified Francis-Harvey-Yates Procedure

(a) Ashing. Introduce the substance to be analyzed into a 150 cc. nickel crucible. Add 5 cc. of N potassium hydroxide, 0.1 cc. of 20% sucrose solution (to prevent formation of bromate) and 10 cc. of distilled water. Mix well. Evaporate to dryness on a water bath, heat at 150°–160° C. for one hour, and ignite in a muffle furnace for one hour at 475°–485°. Cool. Add 20 cc. of water, break up the carbonized mass with a glass rod, digest on the water bath for two to three minutes, and decant through a filter paper (9 cm. Whatman, previously washed with hot distilled water) into a second, clean nickel crucible. Repeat the extraction, and combine the extracts. Place the filter paper in the first crucible, moisten with water and 1 cc. of N potassium hydroxide, evaporate to dryness, heat at 150°–160° for 15 min., and at 475°–485° for one hour. Cool; extract the residue as before. Evaporate the combined extracts to dryness, heat at the two specified temperatures for 15 and 10 min. respectively, cool, dissolve in 10 cc. of water, evaporate, and repeat the short ignition. To remove the last traces of organic matter, dissolve in 5 cc. of water, add a small crystal of potassium nitrate (about 1.5 mg.), evaporate, and repeat the ignition.

Treat the residue with a little water and filter (7 cm. Whatman, treated as before) into a third crucible. Wash the crucible and filter with successive portions of water to make the filtrate up to 30 cc. Evaporate to dryness and ignite at the two temperatures for 15 and 5 min. respectively. (If the material has to be kept overnight, the third crucible should be of platinum.)

(b) Oxidation. Dissolve the contents of the crucible in 3.0 cc. of distilled water, accurately measured, and transfer to a 100 cc. Erlenmeyer flask. Wash the crucible with two additional 2.0-cc. portions of water. The flask is fitted with a two-holed rubber stopper (rubber does not seem to affect the results in this particular procedure). Incline the flask at an angle of 60° to ensure maximum aeration. The stopper carries two tubes, one slightly drawn out and reaching the bottom of the inclined flask, the other bent to 60° and connected to a small bubbler containing 1 cc. of 10% potassium iodide and four drops of 1% starch. Run 2.5 cc. of concentrated sulphuric acid down the inside of the flask, with constant shaking and cooling under the tap. This must be done very slowly and should take at least 10 min. Then run in 4 cc. of chromic acid-sulphuric acid mixture (20 gm. of chromic acid, 40 cc. of concentrated sulphuric acid (sp. gr. 1.84) and 120 cc. of distilled water). Insert the stopper and aerate the contents for one hour. Change the receiver and aerate a further two hours. Titrate the liberated iodine against $N/500$ thiosulphate from a microburette.

Blanks must be determined from time to time, and the figures found subtracted from actual determinations. Usually a perfect blank result is obtained. With one sample of potassium hydroxide a blank of 0.003 mg. of bromine was found.

This method seems to give accurate results for amounts of bromine over the range 0.003–0.9 mg., as the figures in Tables I and II indicate.

TABLE I

ESTIMATION OF KNOWN AMOUNTS OF BROMINE

No.	Iodine present, mg.	Chlorine present, mg.	Bromine present, mg.			Bromine found, mg.	Difference, mg.
			Inorg.	Org.	Total		
1	0.044	2.39	0.001	0.002	0.003	0.003	0.000
2	0.044	5.46	0.001	0.002	0.003	0.004	+0.001
3	0.022	1.19	0.009	0.011	0.020	0.021	+0.001
4	0.022	1.19	0.009	0.011	0.020	0.020	0.000
5	0.044	2.39	0.019	0.022	0.041	0.040	−0.001
6	0.044	5.46	0.038	0.040	0.078	0.076	−0.002
7	0.044	5.46	0.093	0.099	0.192	0.190	−0.002
8	0.044	5.46	0.186	0.203	0.389	0.387	−0.002
9	0.044	5.46	0.190	0.200	0.390	0.390	0.000
10	0.088	5.46	0.376	0.409	0.785	0.781	−0.004
11	0.088	5.46	0.428	0.467	0.895	0.877	−0.018
12	0.088	5.46	0.560	0.609	1.169	0.975	−0.194
13	0.044	5.46	0.929	1.008	1.937	1.466	−0.471

TABLE II

RECOVERY OF ADDED BROMINE

Material analyzed	Bromine found, mg.	Bromine added, mg.		Total bromine, mg.		Difference, mg.
		Inorg.	Org.	Estd.	Found	
Wheat	0.0024	0.0171	—	0.0195	0.0197	+0.0002
Carrot	0.0980	0.0085	—	0.1065	0.1056	−0.0009
Beef blood	0.0144	0.0190	0.0200	0.0534	0.0528	−0.0006
Thymus	0.0064	0.0190	0.0200	0.0454	0.0448	−0.0006
Urine	0.0688	0.0190	0.0200	0.1078	0.1072	−0.0006

The results obtained by the use of this method are given in Part II. The method has now been used continuously for 10 months. A set of checks with known quantities of bromine (dibromtyrosine, or potassium bromide, or both together) have been run about once a week throughout, with uniformly consistent results, typified in Table III. It will be seen that the results tend to be too low by a negligibly small amount.

TABLE III

TYPICAL CHECKS OF PROCEDURE OVER A 10 MONTH PERIOD

Date	Iodine present, mg.	Chlorine present, mg.	Bromine present, mg.			Bromine found, mg.	Difference, mg.
			Inorg.	Org.	Total		
June 11	0.0107	—	0.0332	—	0.0332	0.0327	−0.0005
July 30	—	—	0.0405	—	0.0405	0.0392	−0.0013
Oct. 21	—	—	0.0580	—	0.0580	0.0576	−0.0004
Nov. 20	—	0.120	—	0.0190	0.0190	0.0184	−0.0006
Dec. 27	0.0210	—	0.0819	0.0102	0.0921	0.0912	−0.0009
Jan. 8	—	0.200	0.0432	—	0.0432	0.0422	−0.0010
Feb. 17	0.0150	—	0.0170	—	0.0170	0.0160	−0.0010
Mar. 25	—	—	0.0871	—	0.0871	0.0864	−0.0007

Since undoubtedly many of the analyses of plant and animal material recorded in the literature are based upon inaccurate methods, such results must be accepted with reservations, and it seems desirable to attempt to evaluate the procedures adopted by other investigators through experience gained in establishing the accuracy of the method now reported.

All procedures used in analyzing normal material have consisted essentially of an initial conversion of organic to inorganic bromine by some process of ashing or fusion, and subsequent estimation by titrimetric or colorimetric methods. As pointed out especially by Pincussen (75) and Olszycka (71) and confirmed by the present writer, the initial stage calls for great care. Fusion to a dry ash leads to indeterminate and possibly large loss of bromine. Fusion in presence of alkali or of acid must be thoroughly controlled, to give accurate figures. Applying these criteria the following appears to be legitimate appraisal of the data quoted in this paper from earlier investigators.

Justus (46) fused the material to a dry ash, liberated bromine with nitrous acid and took it up with chloroform. His results are extremely high, owing to the uncontrolled use of nitrous acid.

Lobat (55) fused the material to a dry ash, liberated bromine, and measured the color developed with fluorescein. His results were usually negative, and his method undoubtedly was not sufficiently delicate. The same criticism applies to the methods of Baubigny (5), and Serbescu and Buttu (82).

Walter's method (92) applies only to protein-free filtrates from blood. Auric chloride is added and the color developed is matched against a standard. Comparable but not absolute accuracy is obtained with this method (*cf.* Malamud *et al.* (58)).

Damiens (18–23) used fusion with alkali and the development of a specific color with fuchsin, first described by Denigès and Chelle (26). His method is probably moderately accurate (*cf.* Olszycka (71)).

Bernhardt and Ucko (7) fused the material with alkali, and employed a modification of Damien's colorimetric procedure. According to Olszycka (71) the method is inaccurate and the results it gives are too high.

Roman (77) used fusion with alkali, liberation of bromine by nitric acid-hydrogen peroxide, extraction with chloroform, and titration with thiosulphate. His method has been adversely criticized by Fleischhacker and Scheiderer (32, 33) and by Hahn (41); undoubtedly many results obtained by it are too high.

Behr, Palmer and Clarke (6) used fusion with alkali, and liberated free bromine by phosphoric acid-potassium permanganate mixture, extracted it with carbon tetrachloride, and titrated with thiosulphate. The method has not yet been tested by other investigators, but is possibly open to the same type of criticism that applies to the method of Roman.

Guillaumin and Merejkowsky (40) oxidized the sample in acid-permanganate solution, and then followed a method with fuchsin based upon that of Damiens. The accuracy of their results is probably similar to his.

Francis and Harvey's method (34) has already been criticized. It gives results that are too low.

Dixon (27) fuses the material with alkali, and converts to bromate, which is estimated iodometrically. This type of procedure, carried out under carefully controlled conditions, has been found accurate by Meulen (61) and by Kolthoff (49).

PART II. DISTRIBUTION IN PLANT AND ANIMAL MATERIAL

Introduction

To obtain an understanding of the physiological function of bromine, if it has such a function, it is important to know its distribution in both plant and animal tissues.

The results obtained in the present investigation will first be set out in detail, and then a comparison will be made between these and earlier published data. This will be followed by brief considerations of the possible relationships between the halogens in living tissues, the bromine cycle in nature, and the possible functional significance of bromine.

Experimental Results

The method used throughout this work for bromine determinations is a combination of two methods previously described—a slightly modified procedure of alkali fusion, as used by Francis and Harvey (34) and the oxidation procedure described by Yates (98) as already detailed. In numerous cases individual analyses were controlled by duplicates with varying amounts of material. At no time have difficulties due to the presence of large amounts of chloride been encountered, but in the presence of large amounts of iodide, some of the iodide is oxidized along with the bromide. This difficulty was successfully overcome by reducing the amount of substance taken for each analysis.

A systematic study of the normal bromine content of as great a variety of material as was easily available has been made. A portion of the material used was collected mostly at the Pacific Coast Station of the Biological Board of Canada (at Departure Bay, British Columbia) in the summers of 1913 and 1914 by Dr. A. T. Cameron. These samples have all been kept in stoppered bottles, but, to remove any water that may have been taken up in the long period of storage, each sample was heated in a 100° oven to constant weight. The approximate localities from which these samples were obtained are as follows:

(a) At the Biological Station, Departure Bay, B.C., or at points within half a mile of it.

(b) Near Snake Island, two miles east of *(a)*.

(c) From the sand flats off Protection Island, two miles southeast of *(a)*.

(d) In False Narrows, about eight miles southeast of *(a)*.

(e) At Nanoose, 10 miles northeast of *(a)*.

(f) At North West Bay, 20 miles northeast of *(a)*.

(g) At Belle Chain, 50 miles southeast of *(a)*.
(h) South of Mudge Island, two miles south of *(d)*.
(i) Off Suquash, north of Vancouver Island, B.C.
(j) From Alaskan waters.
(k) From the Canadian Atlantic coast.
(m) From the Marine Biological Station, Plymouth, England.

Dr. W. F. Geddes, of the Dominion Grain Commissioners' Research Laboratory in Winnipeg, kindly prepared especially for these analyses 23 samples of wheat, consisting of No. 1 and 2 Manitoba Hard. These are pooled samples of some 46,000 individual samples from the 1933 and 1934 crops, made up according to 23 different protein-content areas of the three western prairie provinces, as follows:

(n) Alberta.
(o) Saskatchewan.
(p) Manitoba.

The remainder of the plant material was obtained at the following points:

(q) Winnipeg, within a radius of 40 miles.
(r) Carnduff, Saskatchewan.
(s) Delta Manor, Ladner, British Columbia (about four miles from the ocean shore.
(t) California.

With the exception of the cereal grains, all the new material was dried in a 100° oven to constant weight. The cereal grains were analyzed in the fresh condition.

The tissues from rats (pooled samples from five females and five males), rabbits (pooled samples from one female and one male), and dog (female collie), and the other material obtained in the laboratory, were dried in an oven at 100° C. The rabbit thyroid, pituitary and ovary materials are pooled samples from 24 rabbits used in Friedman tests.

The thyroglobulin and thyroid nucleoprotein preparations were obtained from Dr. Cameron. The first-mentioned was prepared by the Oswald procedure, purified by repeated treatment with 1% sodium chloride and ammonium sulphate, dialyzed in distilled water, and precipitated with alcohol.

Of the endocrine products reported on in Table VIII, the Winnipeg material was collected from a local slaughterhouse (Burns and Co.). The hog thyroids and the American samples of pituitary glands were sent to Dr. Cameron by Dr. Frederic Fenger of the Armour Organotherapeutic Laboratories for the purpose of this work. The sheep thyroid preparation was obtained earlier from the same source. The samples labelled "Commercial" were purchased in the usual way.

On account of the possible interrelation of halogens, it seemed desirable to include as many iodine analyses as possible. All the iodine values in Tables IV and VI are taken from Dr. Cameron's papers (11–14). A certain number of iodine analyses have been carried out by the present writer using Kendall's method (47).

The results obtained are given in Tables IV to X.

TABLE IV
MARINE ALGAE

Family and species	Where obtained	Part examined	Amount taken dry material, gm.	Bromine found		Iodine %
				mg.	%	
Phaeophyceae						
Encoliaceae						
Scytosiphon lomentarius	(*e*)	Plant	0.3062	0.248	0.081	
			0.4178	0.331	0.081	
				Mean	0.081	0.014
Laminariaceae						
Nereocystis lütkeana	(*g*)	Frond	0.1071	0.153	0.143	
			0.2438	0.349	0.143	
				Mean	0.143	0.098
Macrocystis pyrifera	(*i*)	Frond	0.06555	0.052	0.079	
			0.1248	0.101	0.081	
				Mean	0.080	0.020
Fucaceae						
Fucus furcatus	(*a*)	Plant	0.0864	0.050	0.058	
			0.1954	0.116	0.059	
				Mean	0.0585	0.042
Rhodophyceae						
Rhodophylidaceae						
Euthora fructiculosa	(*a*)	Plant	0.0961	0.083	0.087	
			0.2045	0.179	0.088	
				Mean	0.0875	0.053
Dumontiaceae						
Constantinea sitchensis	(*a*)	Plant	0.10535	0.046	0.043	
			0.23385	0.103	0.044	
				Mean	0.0435	0.019

TABLE V
LAND PLANTS

Family and species	Where obtained	Part examined	Water, %	Amount taken dry material, gm.	Bromine found	
					mg.	%
Gramineae						
Phleum pratense	(*s*)	Leaves and stems	38.24	0.5072	0.0096	0.0019
(Timothy)				0.5076	0.0096	0.0019
					Mean	0.0019
Arrhenatherum elatius	(*q*)	Leaves and stems	56.55	0.5084	0.0040	0.0008
(Tall oat grass)				0.5242	0.0048	0.0009
					Mean	0.00085
Zea mays	(*q*)	Stem	83.62	0.5023	0.0184	0.0037
(Indian corn)				0.5014	0.0184	0.0037
					Mean	0.0037
		Leaves	80.61	0.5166	0.0136	0.0026
				0.5109	0.0136	0.0027
					Mean	0.00265
		Fruit	79.92	0.5096	0.0032	0.0006
				0.5098	0.0032	0.0006
					Mean	0.0006
		Root	—	0.5049	0.0048	0.0010
				0.5021	0.0032	0.0006
					Mean	0.0008

TABLE V—*Continued*

LAND PLANTS

Family and species	Where obtained	Part examined	Water, %	Amount taken fresh material, gm.	Bromine found mg.	Bromine found %
Gramineae—*Continued*						
Triticum vulgare var. Ceres (Wheat)	(*q*)	Root	—	0.5023	0.0032	0.0006
				0.5094	0.0032	0.0006
					Mean	0.0006
		Straw	—	0.5098	0.0048	0.0009
				0.5088	0.0048	0.0009
					Mean	0.0009
		Grain	—	0.5052	0.0008	0.0001
				0.6059	0.0008	0.0001
					Mean	0.0001
Triticum durum—1933	(*r*)	Grain	—	0.6151	0.0008	0.0001
				0.5123	0.0008	0.0001
					Mean	0.0001
—1935			—	0.6031	0.0024	0.0004
				0.5049	0.0016	0.0003
					Mean	0.00035
Secale cereale (Rye)	(*r*)	Grain	—	0.5282	0.0032	0.0006
				0.6057	0.0032	0.0005
					Mean	0.00055
No. 1 and 2 Manitoba Hard wheat	(*n*)	Grain	—	0.7075	0.0008	0.0001
				0.5543	0.0008	0.0001
					Mean	0.0001
			—	0.7501	0.0024	0.0003
				0.5470	0.0024	0.0004
					Mean	0.00035
			—	0.7135	0.0008	0.0001
				0.5107	0.0008	0.0002
					Mean	0.00015
			—	0.7572	0.0008	0.0001
				0.5590	0.0008	0.0002
					Mean	0.00015
			—	0.6637	0.0008	0.0001
				0.6123	0.0016	0.0003
					Mean	0.0002
			—	0.6051	0.0024	0.0004
				0.7534	0.0024	0.0003
					Mean	0.00035
			—	0.7085	0.0016	0.0002
				0.6105	0.0016	0.0003
					Mean	0.00025
			—	0.7142	0.0008	0.0001
				0.5185	0.0008	0.0002
					Mean	0.00015
			—	0.7397	0.0024	0.0003
				0.6169	0.0008	0.0001
					Mean	0.0002
	(*o*)		—	0.7523	0.0008	0.0001
				0.6339	0.0032	0.0005
					Mean	0.0003
			—	0.6116	0.0024	0.0004
				0.7577	0.0024	0.0003
					Mean	0.00035
			—	0.6243	0.0008	0.0001
				0.7082	0.0016	0.0002
					Mean	0.00015
			—	0.7562	0.0016	0.0002
				0.6149	0.0024	0.0004
					Mean	0.0003

TABLE V—*Continued*

LAND PLANTS

Family and species	Where obtained	Part examined	Water, %	Amount taken fresh material, gm.	Bromine found	
					mg.	%
Gramineae—*Concluded*						
No. 1 and 2 Manitoba Hard wheat—*Concluded*						
			—	0.6595	0.0032	0.0005
			—	0.6093	0.0016	0.0003
			—	0.6112	0.0024	0.0004
			—	0.5548	0.0024	0.0004
				0.7544	0.0024	0.0003
					Mean	0.00035
			—	0.6209	0.0024	0.0004
				0.7167	0.0032	0.0004
					Mean	0.0004
			—	0.7466	0.0024	0.0003
	(p)		—	0.8100	0.0024	0.0003
			—	0.6820	0.0056	0.0008
			—	0.7608	0.0056	0.0007
			—	0.7500	0.0080	0.0011
				Dry material		
Polygonaceae						
Rheum rhaponticum Rhubarb	*(q)*	Leaves	92.68	0.5061	0.0048	0.0009
				0.5067	0.0048	0.0009
					Mean	0.0009
Chenopodiaceae						
Beta rapa (Red beet)	*(q)*	Leaves	88.11	0.3049	(None)	<0.00025
				0.5053	(None)	<0.00015
					Mean	<0.00015
		Root	84.36	0.4118	(None)	<0.0002
				0.5073	(None)	<0.00015
					Mean	<0.00015
Cruciferae						
Brassica rapa (Turnip)	*(s)*	Leaves	83.23	0.5088	0.0216	0.0042
				0.5077	0.0216	0.0043
					Mean	0.00425
		Root	89.49	0.5058	0.0120	0.0024
				0.5052	0.0120	0.0024
					Mean	0.0024
Brassica oleracea (Cabbage)	*(s)*	Leaves	89.16	0.5176	0.0128	0.0025
				0.5052	0.0128	0.0025
					Mean	0.0025
	(q)		89.50	0.5055	0.0008	0.0002
				0.5059	0.0008	0.0002
					Mean	0.0002
Leguminosae						
Trifolium pratense (Red clover)	*(s)*	Leaves and stems	75.36	0.4991	0.0080	0.0016
				0.5012	0.0080	0.0016
					Mean	0.0016
Trifolium pratense (Red clover)	*(s)*	Root	64.40	0.5046	0.0032	0.0006
				0.5035	0.0048	0.0009
					Mean	0.00075
Melilotus alba (Sweet clover)	*(q)*	Leaves and stems	72.75	0.5109	0.0016	0.0003
				0.5070	0.0016	0.0003
					Mean	0.0003
Rosaceae						
Malus communis (Apple)	*(s)*	Fruit	87.57	0.5089	0.0016	0.C003
				0.5021	0.0016	0.0003
					Mean	0.0003

TABLE V—*Continued*

LAND PLANTS

Family and species	Where obtained	Part examined	Water, %	Amount taken dry material, gm.	Bromine found	
					mg.	%
Rosaceae—*Concluded*						
Pyrophorum communis	*(s)*	Fruit	88.82	0.4997	0.0032	0.0006
(Pear)				0.4993	0.0032	0.0006
					Mean	0.0006
Rubus idaeus	*(s)*	Fruit	86.63	0.5133	0.0048	0.0009
(Raspberry)				0.5116	0.0048	0.0009
					Mean	0.0009
Prunus persica	*(t)*	Fruit	88.06	0.5042	(None)	<0.00015
(Peach)				0.5024	(None)	<0.00015
					Mean	<0.00015
Citrus aurantium	*(t)*	Fruit-edible	89.86	0.5103	0.0032	0.0006
(Orange)				0.5091	0.0032	0.0006
					Mean	0.0006
		Fruit peel	75.42	0.5000	0.0032	0.0006
				0.5020	0.0016	0.0003
					Mean	0.00045
Citrus decumana	*(t)*	Fruit-edible	91.74	0.5121	0.0048	0.0009
(Grapefruit)				0.5089	0.0048	0.0009
					Mean	0.0009
		Fruit peel	80.96	0.5000	0.0016	0.0003
				0.5005	Trace	Trace
					Mean	<0.0003
Vitaceae						
Vitis vinifera	*(s)*	Fruit	87.58	0.5085	0.0048	0.0010
(Grapes)				0.5112	0.0064	0.0012
					Mean	0.0011
	(t)	Fruit	85.37	0.5090	(None)	<0.00015
				0.5083	(None)	<0.00015
					Mean	<0.00015
Umbelliferae						
Daucus carota	*(q)*	Leaves and stems	80.54	0.2518	(None)	<0.0003
(Carrot)				0.4050	(None)	<0.0002
					Mean	<0.0002
		Root	89.48	0.3082	(None)	<0.00025
				0.4086	(None)	<0.0002
					Mean	<0.0002
	(s)	Leaves and stems	77.71	0.5016	0.0981	0.0195
				0.5024	0.0984	0.0196
					Mean	0.01955
		Root	89.58	0.5095	0.0184	0.0036
				0.5123	0.0184	0.0036
					Mean	0.0036
Apium vapacium	*(s)*	Leaves and stems	87.16	0.5074	0.0880	0.0173
(Celery)				0.5053	0.0912	0.0180
					Mean	0.01765
Pastinaca sativa	*(q)*	Leaves	80.25	0.5080	0.0128	0.0025
(Parsnip)				0.5056	0.0128	0.0025
					Mean	0.0025
		Root	77.13	0.5072	0.0064	0.0013
				0.5055	0.0064	0.0013
					Mean	0.0013
Solanaceae						
Solanum tuberosum	*(q)*	Leaves	84.26	0.3077	(None)	<0.00025
(Potato)				0.40485	(None)	<0.0002
					Mean	<0.0002
		Root	80.21	0.2963	(None)	<0.00025
				0.3436	(None)	<0.00025
					Mean	<0.00025

TABLE V—*Concluded*

LAND PLANTS

Family and species	Where obtained	Part examined	Water, %	Amount taken dry material, gm.	Bromine found	
					mg.	%
Solanaceae—*Concluded*						
Lycopersicon esculentum	(*q*)	Fruit	93.90	0.5045	0.0016	0.0003
(Tomato)				0.5033	0.0016	0.0003
					Mean	0.0003
	(*s*)	Fruit	95.22	0.5027	0.0064	0.0013
				0.5069	0.0080	0.0015
					Mean	0.0014
Compositae						
Lactuca sativa	(*q*)	Leaves	94.51	0.5017	0.0096	0.0019
(Lettuce)				0.5012	0.0096	0.0019
					Mean	0.0019
Cucurbitaceae						
Cucurbita sativus	(*q*)	Fruit	95.44	0.5024	0.0200	0.0040
(Cucumber)				0.5033	0.0200	0.0040
					Mean	0.0040

TABLE VI

MARINE ANIMALS

Family and species	Where obtained	Part examined	Amount taken dry material, gm.	Bromine		Iodine, %
				mg.	%	
Phylum Porifera						
Monaxonida						
Myxilla parasitica	(*b*)	Animal	0.0836	0.020	0.024	
			0.2342	0.057	0.025	
				Mean	0.0245	0.010
Esperella adhaerens	(*b*)	Animal	0.08625	0.017	0.019	
			0.20475	0.040	0.020	
				Mean	0.0195	0.015
Hexactinellida						
Bathydorus dawsonii	(*b*)	Animal	0.0563	0.0040	0.0071	
			0.0758	0.0056	0.0074	
				Mean	0.00725	0.009
Rhabdocalyptus dowlingii	(*b*)	Animal	0.08725	0.0016	0.0018	
			0.16445	0.0032	0.0019	
				Mean	0.00185	0.014
Phylum Coelenterata						
Hydrozoa						
Aequorea forskalia	(*d*)	Animal	0.18725	0.214	0.114	
			0.0759	0.086	0.113	
				Mean	0.1135	0.000
Scyphozoa						
Aurelia flavidula	(*d*)	Animal	0.0856	0.164	0.192	
			0.0847	0.163	0.193	
				Mean	0.1925	0.000
Actinozoa						
Matridium marginatum	(*a*)	Animal	0.08445	0.030	0.035	
			0.1737	0.062	0.036	
				Mean	0.0355	0.000
Ctenophora						
Pleurobrachia (sp. ?)	(*a*)	Animal	0.07815	0.148	0.189	
			0.1174	0.212	0.189	
				Mean	0.189	0.000

TABLE VI—*Continued*

MARINE ANIMALS

Family and species	Where obtained	Part examined	Amount taken dry material, gm.	Bromine, mg.	Bromine, %	Iodine, %
(Verticillate fan coral) ? *Caligorgia*	(*j*)	Whole coral	0.2042	0.718	0.352	
			0.40455	1.442	0.356	
				Mean	0.354	0.057
		Skeleton	0.04505	0.394	0.874	
			0.07785	0.683	0.878	
				Mean	0.876	0.099
Phylum Vermes						
Eunicea						
Diopatra (? californica)	(*h*)	Inner tubes	0.10415	0.121	0.116	
			0.2001	0.239	0.119	
				Mean	0.1175	0.122
		Interm. tubes	0.10155	0.123	0.121	
			0.2057	0.251	0.122	
				Mean	0.1215	0.128
		Outer tubes	0.30455	0.121	0.039	
			0.4027	0.158	0.039	
				Mean	0.039	0.041
Chaetopterida						
Chaetopterus (sp. ?)	(*c*)	Inner tubes	0.1035	0.330	0.318	
			0.0714	0.230	0.320	
				Mean	0.319	0.450
		Interm. tubes	0.1257	0.297	0.236	
			0.18175	0.416	0.229	
				Mean	0.2325	0.333
		Outer tubes	0.2032	0.305	0.150	
			0.1505	0.229	0.152	
				Mean	0.151	0.212
		Tube ends	0.3024	0.145	0.048	
			0.1067	0.049	0.046	
				Mean	0.047	0.096
Sabellidae						
Eudistylia gigantea	(*d*)	Worm	0.3080	0.062	0.020	
			0.4007	0.078	0.019	
				Mean	0.0195	0.030
		Inner tube	0.0723	0.029	0.040	
			0.0507	0.021	0.040	
				Mean	0.040	0.616
		Interm. tube	0.05425	0.020	0.036	
			0.0729	0.027	0.037	
			0.20805	0.078	0.038	
				Mean	0.037	0.606
	(*a*)	Outer tube	0.1054	0.086	0.082	
			0.14025	0.116	0.083	
			0.2044	0.171	0.084	
				Mean	0.083	0.587
		Tube	0.0899	0.062	0.069	
			0.1734	0.119	0.068	
				Mean	0.0685	0.572
Bispira polymorpha	(*a*)	Tube	0.07615	0.103	0.136	
			0.1406	0.190	0.135	
				Mean	0.1355	0.656
			0.05545	0.074	0.134	
			0.1041	0.141	0.134	
				Mean	0.134	0.698
Phoronida						
Phoronopsis harmeri	(*c*)	Outer tube	0.3103	0.0061	0.0020	
			0.45745	0.0086	0.0019	
				Mean	0.00195	0.009

TABLE VI—*Concluded*

MARINE ANIMALS

Family and species	Where obtained	Part examined	Amount taken dry material, gm.	Bromine, mg.	Bromine, %	Iodine, %
Phylum Mollusca						
Schizotherus nuttali	*(c)*	Dermis	0.2028	0.205	0.101	
(Horse clam)		of foot	0.17275	0.179	0.104	
				Mean	0.1025	0.092
	(e)		0.2102	0.215	0.102	
			0.30445	0.301	0.099	
				Mean	0.1005	0.103
Mytilus edulis (Mussel)	*(a)*	Byssus	0.2021	0.274	0.135	0.042
Polynices lewisii	*(c)*	Opercula	0.17155	0.660	0.385	
(Whelk)			0.06755	0.256	0.379	
				Mean	0.382	0.030
Phylum Chordata						
Sub-phylum Tunicata						
Tethyidae						
Pyura haustor	*(h)*	Test	0.5082	0.502	0.099	
			0.5042	0.493	0.098	
				Mean	0.0985	0.216
	(h)		0.50695	0.809	0.160	0.247
	(e)		0.5020	0.278	0.055	
			0.5006	0.251	0.051	
				Mean	0.053	0.027
Phylum Chordata						
Sup-phylum tunicata						
Tethyidae						
Tethyum igaboja	*(b)*	Test	0.1397	0.323	0.231	
			0.2103	0.472	0.225	
				Mean	0.228	0.169
Styelidae						
Cnemidocarpa joannae	*(a)*		0.2261	0.301	0.133	0.106
Phalusiidae						
Ascidiopsis paratropa	*(f)*		0.2810	0.579	0.206	
			0.1935	0.405	0.209	
				Mean	0.2075	0.010
Phylum Chordata						
Sub-phylum Vertebrata						
Raia clavata	*(m)*	Thyroid	0.1701	0.080	0.047	
			0.1760	0.082	0.046	
				Mean	0.0465	0.438
			0.2032	0.110	0.054	
			0.19085	0.100	0.052	
				Mean	0.053	0.327
			0.2080	0.216	0.104	
			0.22755	0.241	0.105	
				Mean	0.1045	0.283
Scyllium canicula	*(m)*		0.2034	0.102	0.050	
			0.2088	0.107	0.051	
				Mean	0.0505	0.719
			0.2060	0.165	0.080	
			0.22325	0.175	0.078	
				Mean	0.079	1.160
Acanthias vulgaris	*(k)*		0.1631	0.021	0.013	
			0.13675	0.018	0.013	
				Mean	0.013	0.133
Squalus sucklii	*(a)*		0.1119	0.037	0.033	0.216
			0.08445	0.033	0.039	0.185
Hydrolagus collieii	*(a)*	Egg-case	0.20175	0.074	0.037	
			0.15355	0.058	0.038	
				Mean	0.0375	0.029

TABLE VII
MAMMALIAN MATERIAL

Animal	Part examined	Water %	Amount taken dry material, gm.	Bromine found		Calculated to Br in fresh material, %
				mg.	%	
Albino rats	Blood	80.39	0.5090	0.0136	0.0027	
			0.3580	0.0120	0.0033	
				Mean	0.0030	0.00059
	Bone	43.26	0.5093	0.0032	0.0006	0.00034
	Cerebellum	76.74	0.4491	0.0056	0.0012	
			0.4088	0.0056	0.0013	
				Mean	0.00125	0.00028
	Cerebrum	79.62	0.4992	Trace	Trace	
			0.5004	Trace	Trace	
				Mean	Trace	Trace
	Eye	81.02	0.3803	0.0048	0.0013	0.00025
	Heart	77.34	0.5026	0.0072	0.0014	
			0.3804	0.0048	0.0013	
				Mean	0.00135	0.000295
	Large intestine	78.60	0.5027	0.0048	0.0010	0.000215
	Small intestine	78.18	0.5026	0.0032	0.0006	
			0.5026	0.0032	0.0006	
				Mean	0.0006	0.00013
	Kidney	83.77	0.5042	0.0056	0.0011	
			0.5052	0.0056	0.0011	
				Mean	0.0011	0.00018
	Liver	74.97	0.4991	0.0024	0.0005	
			0.5015	0.0032	0.0006	
				Mean	0.00055	0.000125
	Lung	79.32	0.5090	0.0104	0.0020	
			0.5102	0.0096	0.0019	
				Mean	0.00195	0.00039
	Medulla	71.23	0.2833	0.0048	0.0017	0.00050
	Muscle	74.52	0.4979	0.0032	0.0006	
			0.5021	0.0024	0.0005	
				Mean	0.00055	0.000125
	Pancreas	65.19	0.4990	0.0032	0.0006	
			0.5088	0.0040	0.0008	
				Mean	0.0007	0.00024
	Skin	58.68	0.5138	0.0040	0.0008	
			0.5147	0.0048	0.0008	
				Mean	0.0008	0.00033
	Spleen	77.07	0.5003	0.0040	0.0008	0.000185
	Stomach	78.99	0.5090	0.0080	0.0016	
			0.5073	0.0064	0.0013	
				Mean	0.00145	0.00030
	Testes	85.81	0.5137	0.0091	0.0018	
			0.5138	0.0109	0.0021	
				Mean	0.00195	0.00027
	Uterus	73.55	0.1904	0.0040	0.0021	0.00056
	Hair	Fresh material	10.4965	0.0019	—	0.0004
Rabbits	Adrenal	70.48	0.2918	0.0032	0.0011	0.00032
	Blood	81.71	0.5150	0.0144	0.0028	
			0.5115	0.0144	0.0028	
				Mean	0.0028	0.00051
	Bone	32.72	0.5143	0.0048	0.0009	
			0.5411	0.0048	0.0009	
				Mean	0.0009	0.000605
	Bone marrow	52.62	0.5012	0.0032	0.0006	
			0.5138	0.0032	0.0006	
				Mean	0.0006	0.000285

TABLE VII—*Continued*

MAMMALIAN MATERIAL

Animal	Part examined	Water %	Amount taken dry material, gm.	Bromine found		Calculated to Br in fresh material, %
				mg.	%	
Rabbits *(concluded)*	Cartilage	67.39	0.5063	0.0064	0.0013	
			0.5140	0.0064	0.0013	
				Mean	0.0013	0.00043
	Cerebellum	79.73	0.5054	0.0016	0.0003	
			0.5067	0.0016	0.0003	
				Mean	0.0003	0.00006
	Cerebrum	78.62	0.5096	0.0048	0.0009	0.00019
	Eye	87.68	0.5155	0.0120	0.0023	
			0.5125	0.0120	0.0023	
				Mean	0.0023	0.00028
	Heart	78.16	0.5126	0.0016	0.0003	
			0.5206	0.0016	0.0003	
				Mean	0.0003	0.000065
	Kidney	78.95	0.5112	0.0032	0.0006	
			0.5088	0.0032	0.0006	
				Mean	0.0006	0.00013
	Liver	75.43	0.5179	0.0016	0.0003	
			0.5083	0.0016	0.0003	
				Mean	0.0003	0.00007
	Lung	80.20	0.5049	0.0032	0.0006	
			0.5059	0.0032	0.0006	
				Mean	0.0006	0.00012
	Medulla	66.78	0.5101	0.0016	0.0003	
			0.5135	0.0016	0.0003	
				Mean	0.0003	0.00010
	Muscle	76.74	0.5167	0.0016	0.0003	
			0.5092	0.0016	0.0003	
				Mean	0.0003	0.00007
	Skin	69.05	0.5707	0.0016	0.0003	
			0.5745	0.0016	0.0003	
				Mean	0.0003	0.00009
	Spleen	77.96	0.4919	0.0032	0.0006	0.00013
	Stomach	80.51	0.5033	0.0064	0.0013	
			0.5055	0.0064	0.0013	
				Mean	0.0013	0.00025
	Testes	79.91	0.5105	0.0072	0.0014	
			0.5073	0.0056	0.0011	
				Mean	0.00125	0.00024
	Thymus	51.78	0.5136	0.0016	0.0003	
			0.3618	0.0016	0.0004	
				Mean	0.00035	0.00014
	Uterus	80.26	0.5022	0.0224	0.0045	
			0.5149	0.0232	0.0045	
				Mean	0.0045	0.00089
	Hair	Fresh	0.5114	0.0032	—	0.0006
		material	0.5115	0.0032	—	0.0006
				Mean	—	0.0006
	Ovary	52.12	0.4780	0.0072	0.0015	0.00072
	Pituitary	74.21	0.2562	0.0040	0.0016	0.00041
	Thyroid	71.30	0.1077	0.0032	0.0030	
			0.3549	0.0104	0.0029	
				Mean	0.00295	0.00083
Dog 1	Adrenal	63.85	0.4993	0.0032	0.0006	0.000215
	Bladder bile	53.49	0 5034	0 0016	0.0003	
			0.4993	0.0016	0.0003	
				Mean	0.0003	0.00014

TABLE VII—*Continued*
MAMMALIAN MATERIAL

Animal	Part examined	Water %	Amount taken dry material, gm.	Bromine found mg.	Bromine found %	Calculated to Br in fresh material, %
Dog 1 *(continued)*	Blood	76.21	0.5018	0.0184	0.0037	
			0.5023	0.0200	0.0040	
				Mean	0.00385	0.000905
	Bone	22.67	0.5067	0.0040	0.0008	
			0.5016	0.0040	0.0008	
				Mean	0.0008	0.00062
	Bone marrow	33.81	0.5100	0.0048	0.0009	
			0.4228	0.0048	0.0011	
				Mean	0.0010	0.00066
	Cerebellum	79.03	0.5083	0.0032	0.0006	
			0.5115	0.0040	0.0008	
				Mean	0.0007	0.000145
	Cerebrum	79.37	0.5118	0.0048	0.0009	
			0.5077	0.0040	0.0009	
				Mean	0.0009	0.000165
	Eye	88.25	0.5013	0.0552	0.0110	
			0.5037	0.0512	0.0102	
				Mean	0.0106	0.001245
	Gall bladder	68.07	0.5075	0.0040	0.0008	0.000255
	Heart	76.06	0.5029	0.0040	0.0008	
			0.5047	0.0040	0.0008	
				Mean	0.0008	0.00019
	Large intestine	78.80	0.5066	0.0096	0.0019	
			0.5054	0.0112	0.0022	
				Mean	0.00205	0.000425
	Small intestine	81.37	0.5012	0.0096	0.0019	
			0.5033	0.0096	0.0019	
				Mean	0.0019	0.000355
	Kidney	79.03	0.5123	0.0168	0.0033	
			0.5083	0.0168	0.0033	
				Mean	0.0033	0.00069
	Liver	77.62	0.5006	0.0104	0.0021	
			0.5006	0.0104	0.0021	
				Mean	0.0021	0.00047
	Lung	78.98	0.5090	0.0104	0.0020	
			0.5082	0.0104	0.0020	
				Mean	0.0020	0.00042
	Muscle	74.02	0.5074	0.0016	0.0003	
			0.5087	0.0016	0.0003	
				Mean	0.0003	0.00008
	Ovary	72.34	0.1921	0.0024	0.0012	0.00033
	Pancreas	74.54	0.5080	0.0048	0.0009	
			0.5054	0.0040	0.0008	
				Mean	0.00085	0.000205
	Pituitary	79.88	0.0192	(None)	<0.004	<0.0008
	Skin	47.76	0.5103	0.0096	0.0019	
			0.5203	0.0104	0.0020	
				Mean	0.00195	0.00099
	Spleen	79.17	0.5101	0.0120	0.0024	
			0.5025	0.0120	0.0024	
				Mean	0.0024	0.00050
	Stomach	79.12	0.5079	0.0144	0.0028	
			0.5120	0.0128	0.0025	
				Mean	0.00265	0.00054
	Thymus	69.53	0.5104	0.0072	0.0014	
			0.2696	0.0040	0.0015	
				Mean	0.00145	0.000425

TABLE VII—*Continued*

MAMMALIAN MATERIAL

Animal	Part examined	Water %	Amount taken dry material, gm.	Bromine found		Calculated to Br in fresh material, %
				mg.	%	
Dog 1 *(concluded)*	Thyroid	73.08	0.4180	0.0184	0.0044	
			0.2577	0.0104	0.0040	
				Mean	0.0042	0.00113
	Uterus	77.04	0.4211	0.0080	0.0019	0.000435
	Hair	Fresh	0.5058	0.0048	—	0.0009
		material	0.5105	0.0032	—	0.0006
				Mean	—	0.00075
Dog 2	Thyroid	—	0.2308	0.0104	0.0045	
			0.3625	0.0152	0.0042	
				Mean	0.00435	—

TABLE VIII

NORMAL ENDOCRINE MATERIAL FROM VARIOUS SOURCES

Material	Animal	Source	Water, %	Amount taken dry material, gm.	Bromine found		Calculated to Br in fresh material, %	Iodine dry material, %
					mg.	%		
Thyroid	Cattle	Winnipeg	79.21	0.10575	0.0048	0.0045		0.061
				0.2061	0.0096	0.0046	—	0.063
					Mean	0.00455	0.000935	0.062
		Commercial	—	0.2969	0.029	0.009		0.430
				0.4074	0.036	0.009		
					Mean	0.009	—	0.430
	Hog	Northern U.S.A.	—	0.2037	0.094	0.046		0.282
				0.29955	0.131	0.044		0.285
					Mean	0.045	—	0.2835
		Central U.S.A.	—	0.20145	0.074	0.036		0.373
				0.29855	0.105	0.035		0.373
					Mean	0.0355	—	0.373
		Southern U.S.A.	—	0.2038	0.069	0.033		0.667
				0.4039	0.144	0.035		0.668
					Mean	0.034	—	0.6675
	?	Commercial	—	0.22265	0.050	0.022		0.151
				0.42125	0.091	0.022		
					Mean	0.022	—	0.151
	Sheep	U.S.A.	—	0.22325	0.045	0.020		0.358
				0.42315	0.086	0.020		
					Mean	0.020	—	0.358
Pituitary ant. lobe	Cattle	Winnipeg	75.94	0.1126	0.0032	0.0029		
				0.3575	0.0104	0.0029		
					Mean	0.0029	0.00070	—
Pituitary whole gland	Cattle	U.S.A.	—	0.39975	0.0264	0.0066		0.014
				0.50225	0.0360	0.0071		0.013
					Mean	0.00685	—	0.0135
Pituitary post. lobe	Cattle	Winnipeg	77.97	0.1084	0.0032	0.0030		
				0.35885	0.0145	0.0040		
					Mean	0.0035	0.00077	—
		U.S.A.	—	0.4020	0.0038	0.0009		Trace
				0.5027	0.0045	0.0009		
					Mean	0.0009	—	Trace

TABLE VIII—*Concluded*

NORMAL ENDOCRINE MATERIAL FROM VARIOUS SOURCES

Material	Animal	Source	Water, %	Amount taken dry material, gm.	Bromine found		Calculated to Br in fresh material, %	Iodine dry material, %
					mg.	%		
Adrenal medulla	Cattle	Winnipeg	78.97	0.42535	0.0080	0.0019	0.00040	—
Adrenal cortex	Cattle	Winnipeg	77.27	0.42675	0.0032	0.0008		
				0.5029	0.0048	0.0009		
					Mean	0.00085	0.00018	—
			—	0.3353	(None)	<0.0003		
				0.11385	(None)	<0.0008		
					Mean	<0.0003	—	—
			—	0.5118	0.0096	0.0019		
				0.4023	0.0080	0.0020		
					Mean	0.00195	—	—
Ovarian residue	?	Commercial	—	0.4044	0.0064	0.0016		0.014
				0.5127	0.0080	0.0016		
					Mean	0.0016	—	0.014
Corpora lutea	?	Commercial	—	0.40715	0.0064	0.0016		0.014
				0.5021	0.0064	0.0013		
					Mean	0.00145	—	0.014

TABLE IX

ANALYSIS OF THYROID FRACTIONS

Material	Water, %	Amount taken dry material, gm.	Bromine found		Calculated to Br in fresh material, %	Iodine dry material, %
			mg.	%		
Thyroid—normal (beef)	74.08	0.11245	0.0040	0.0036		0.256
		0.21005	0.0096	0.0046		0.256
			Mean	0.0041	0.00106	0.256
residue left after extn. with 1% NaCl	82.96	0.1075	0.0040	0.0037		0.042
		0.2047	0.0088	0.0043		0.044
			Mean	0.0040	0.00068	0.043
—normal (beef)	—	0.15535	0.0064	0.0041		
		0.3565	0.0160	0.0045		
			Mean	0.0043	—	—
residue left after extn. with 1% NaCl	—	0.1537	0.0024	0.0016		
		0.3521	0.0072	0.0020		
			Mean	0.0018	—	—
Thyroid—normal (hog)	75.17	0.1020	0.0144	0.0141		0.199
		0.31185	0.0456	0.0146		0.198
			Mean	0.01435	0.00356	0.1985
residue left after extn. with 1% NaCl	85.26	0.11445	0.0072	0.0063		0.077
		0.3181	0.2008	0.0065		0.084
			Mean	0.0064	0.00094	0.0805
Thyroglobulin, pure, 1926	—	0.3192	0.066	0.021		0.619
(beef)		0.41855	0.088	0.021		
			Mean	0.021	—	0.619
	—	0.3216	0.048	0.015		0.634
		0.4195	0.068	0.016		0.636
			Mean	0.0155	—	0.635
goitrous	—	0.3215	0.0080	0.0023		0.282
		0.48185	0.0112	0.0023		0.276
			Mean	0.0023	—	0.279

TABLE IX—*Concluded*

ANALYSIS OF THYROID FRACTIONS

Material	Water, %	Amount taken dry material, gm.	Bromine found		Calculated to Br in fresh material, %	Iodine dry material, %
			mg.	%		
Thyroglobulin, pure, 1926	—	0.47025	0.0350	0.0074		0.443
(hog)		0.4921	0.0380	0.0077		
			Mean	0.00755	—	0.443
goitrous	—	0.48975	0.0270	0.0055		0.320
		0.4994	0.0300	0.0060		
			Mean	0.00575	—	0.320
Thyroid, nucleoprotein, 1926	—	0.5037	(None)	<0.00015		0.025
		0.4987	(None)	<0.00015	—	0.023
			Mean	<0.00015	—	0.024

TABLE X

MISCELLANEOUS MATERIAL

Material	Amount taken fresh material, cc.	Bromine found	
		mg.	gm. per 100 cc.
Dairy milk	5.00	0.0048	0.000096
Wheat germ oil	3.00	(None)	<0.00003
	gm.		%
White bread (wrapped)	0.6159	0.0019	0.0003
(unwrapped)	0.6083	0.0040	0.0007
Dog biscuits	0.5143	Trace	Trace
Wheat germ flour	0.5117	0.0008	0.0001
Virginia cigarette tobacco	0.5069	0.0059	0.0012

Comparison of Results with Those of Earlier Investigators

The results of previous authors, in so far as they can be related to dried or fresh plant and animal tissues, are given in Tables XI (plants), and XII and XIII (animal tissues). The figures in Table XI refer to percentage of bromine in dried material, and are all from Damiens' papers, and determined by his method, which probably leads to fairly accurate results. Those in Tables XII and XIII refer to fresh material except where otherwise stated. In these tables, the author's reference is given in parentheses after the percentage figure (or at the heads of columns in Tables XI and XIII), and, where the method used is considered to give unreliable results, these also are placed within parentheses. The present writer's results are inserted for comparison ("N" in parentheses). Certain isolated determinations will be mentioned later in the discussion.

Pincussen (74–77), Zondek and Bier (100–105), Jacobson (45), Ewer (30), Kuranami (51, 52), Urechia and Retezeanu (89, 90), and Tanino (83, 84) used Roman's method (77); Kriwskii (50) used Bernhardt and Ucko's method (7); Pillot (73) used Baubigny's method (5); Chelle (17) used Denigès and Chelle's method (26).

TABLE XI

BROMINE CONTENT OF PLANT MATERIAL

Material	Bromine in dried material, %	
	Damiens (24, 25)	Neufeld (N)
Edible fungi (9 species)	0.00019–0.00362	—
Flowering plants		
Gramineae (Wheat)	0.00021	0.0001–0.0011*
(Rye)	0.00019	0.0005*
(Other cereals—5 species)	Traces–0.00056	—
Liliaceae (Garlic, onion, eschalot, leek)	0.00010–0.00052	—
(Asparagus)	0.00202	—
Urticaceae (Hemp)	0.00021–0.00023	—
Polygonaceae (Rhubarb)	0.00075	0.0009
Chenopodiaceae (Beetroot)	0.00037–0.00055	(None)
Cruciferae (Turnip)	0.00031–0.00089	0.0024
(Cabbage)	0.00045	0.0002 –0.0025
(Radish, cauliflower)	0.00067–0.00083	—
Leguminosae (8 species)	Traces–0.00102	—
Umbelliferae (Celery)	0.00038–0.00047	0.01765
(Carrot)	0.00039	(None)–0.0036
Labiatae (Woundwort)	0.00061	—
Solanaceae (Potato)	0.00027–0.00143	(None)
Compositae (2 species of artichoke)	0.00062–0.00098	—
Fruits		
Peach	Traces–0.00047	(None)
Apple	Traces	0.0003
Raspberry	Traces	0.0009
Orange	0.00032	0.0006
Grape	0.000195	(None)–0.0011
Tomato	0.00095–0.00534	0.0003–0.0014
Ten other species	(None)–0.00071	—
Muskmelon	0.09945	—
Watermelon	0.0262	—
Miscellaneous related material		
Wheat bread	0.00009–0.00061	0.0003–0.0007*
Wheat flour	0.00009–0.00012	—
Wheat germ bread	0.00068	—

**Fresh material.*

TABLE XII

BROMINE CONTENT OF ANIMAL FLUIDS (VARIOUS AUTHORS)

Animal	Mg. per 100 cc.				
	Whole blood	Blood serum	Red corpuscles	Bile	Urine
Rat	(2.2) (34)				
	0.59 (N)	—	—	—	—
Rabbit	0.51 (N)	—	—	—	—
Guinea pig	(2.3) (34)	—	—	—	—
Dog	(0.63–1.71)(7)	(0.71–0.83)(7)	—	0.08 (23)	0.05 (23)
	0.42 (23)	0.60 (23)	—	0.14 (N)	—
	0.91 (N)	—	—	—	—
Hog	(1.3) (34)	0.75–1.25 (28)	—	—	—
Sheep	(1.8) (34)	—	—	—	—
Cow	(11.2) (46)	—	0.90 (N)	—	(2–3) (55)
	(1.7) (34)	—	—	—	—
	(0.95)(50)	—	—	—	—
	(None) (55)	—	—	—	—
	0.52 (19)	—	—	—	—
	0.71–1.33 (N)	—	—	—	—
Horse	(1.05)(50)	—	—	—	—

TABLE XIII

BROMINE CONTENT OF ANIMAL TISSUES (VARIOUS AUTHORS)

Tissue	Mg. per 100 gm. fresh tissue						
	Rat (N)	Rabbit (N)	Dog (N)	Dog (23)	Dog (7)	Hog (28)	Cow (various)
Adrenal	—	0.32	0.215	—	3.3 -5.0	0.368	0.15 (19)
Aorta	—	—	—	—	1.66-2.5	—	—
Bone	0.34	0.605	0.62	—	—	—	—
Bone marrow	—	0.285	0.66	—	—	—	—
Cartilage	—	0.43	—	—	0.77	—	—
Cerebellum	0.28	0.06	0.145	0.20	0.55-0.90	—	—
Cerebrum	Trace	0.19	0.165	—	0.53-1.25	0.191-0.192	(19.3) (46) (0.02) (55)
Eye	0.25	0.28	1.245	—	—	—	—
Fatty depots	—	—	—	—	0.63-0.71	—	—
Gall bladder	—	—	0.255	—	—	—	—
Hair	0.4	0.6	0.7	—	—	—	—
Heart	0.295	0.065	0.19	0.16	0.55-0.63	—	(None) (55)
Large intestine	0.215	—	0.425	—	—	—	—
Small intestine	0.13	—	0.355	—	0.50-0.55	—	(26.8) (46)
Kidney	0.18	0.13	0.69	0.40	0.59-0.83	0.361-0.445	(20.9) (46) (None) (55)
Liver	0.125	0.07	0.47	0.25	0.40-0.63	0.213-0.295	(10.1) (46) (None) (55) (0.559) (45)
Lung	0.39	0.12	0.42	0.40	0.71-0.83	0.397-0.55	(22.9) (46) 0.42 (19)
Medulla	0.50	0.10	—	—	—	—	—
Muscle	0.125	0.07	0.08	0.10	0.50	—	(22.1) (46)
Ovary	—	0.72	0.33	—	—	0.647	(0.836) (45)
Parathyroid	—	—	—	—	—	—	(5.887) (45)
Pancreas	0.24	—	0.205	—	0.55-0.63	0.259-0.265	—
Pituitary	—	0.41	—	—	12.5	0.270	(None-0.23) (55)
Pituitary ant. lobe	—	—	—	—	—	—	(8.716) (45) (15-30) (102)
Pituitary post. lobe	—	—	—	—	—	—	(0.079) (45)
Skin	0.33	0.09	0.99	—	0.37-0.43	—	—
Spleen	0.185	0.13	0.50	0.41	0.63-0.71	—	(21.4) (46) (None) (55)
Stomach	0.30	0.25	0.54	—	0.60-0.77	—	(22.5) (46)
Testes	0.27	0.24	—	0.53	0.63-0.71	0.334	(20.3) (46) (0.85-2.2) (50)
Ad-testes	—	—	—	—	—	—	(0.9-1) (50)
Plexus pampiniformis	—	—	—	—	—	—	(0.95-2.8) (50)
Thymus	—	0.14	0.425	—	—	—	(21.) (46)
Thyroid	—	0.83	1.13	—	0.84-1.45	—	(35.) (46) (6.691) (45) (0.07-3.0) (55)
Trachea	—	—	—	0.20	—	—	—
Uterus	0.56	0.89	0.435	—	—	—	—

Discussion of Results

The contrasted results in Tables XI to XIII, on the whole, show differences to be expected from the criticisms of methods made earlier in this paper. The most extensive series, that of Damiens, tends to be somewhat lower than the present writer's, especially for some of his plant material, presumably from French sources, but is in good general agreement. The figures of Bernhardt and Ucko are slightly but distinctly higher, those of Justus are obviously much too high and those of Lobat much too low.

Marine Plants

Algae

Of the Brown and Red algae examined by the present writer, all contained appreciable amounts of bromine. The highest amount was found in the frond of *Nereocystis lütkeana*. With the exception of the frond of *Macrocystis pyrifera*, the bromine content was always higher than the iodine content; there seems to be no relation between the two.

The number of analyses carried out is insufficient to determine the degree to which environment and selective affinity respectively determine the bromine content of these plants, but comparison with the figures for land plants makes it obvious that environment plays a very important role.

Very little information can be obtained from the literature concerning earlier data on the bromine content of algae. Toller (85) analyzing *Nereocystis lütkeana* from Puget Sound found in two analyses 0.11 and 0.19% of bromine and 0.23 and 0.30% of iodine respectively. The method used was not given. Wolff (94) gives several bromine analyses on the *ash* of European algae. These are, in percentages, *Fucus vesiculosus*, 0.62; *F. servatus*, 1.07; *Halidrys siliquosa*, 0.65; *Laminaria digitata*, 0.80; *L. saccharina*, 0.25. It is probable that much bromine was lost in the ashing of these plants.

Kylin (53), in a systematic study of the iodine content of a large number of algae collected on the west coast of Sweden, carried out qualitative tests for bromine and found it present in most of the algae examined.

Sauvageau (80, 81), being unable to extract the bromine from algae with water before ashing, but readily after, concluded that most of the bromine is present in organic form.

Eel grass

Losana and Croce (56) found 0.061% of bromine and 0.127% of iodine present in *Zostera marina* collected off the northern coast of Africa, while Kylin (53) found no bromine in the same plant collected off the coast of Sweden.

Land Plants

Fungi

The only data in the literature on the bromine content of fungi are those of Damiens and Blaignon (24, 25). It seems of interest that the bromine content tends to be relatively high among the edible varieties.

Flowering Plants

The data for plants given in Tables V and XI are sufficiently complete to show that bromine is present in a measurable amount in the great majority of flowering plants. The amount of bromine present in the environment is undoubtedly a determining factor of the amount present in the plant. This is well illustrated in the case of *Brassica oleracea* (cabbage) grown in the summer of 1935—the Manitoba material has a bromine content of 0.0002%, while the British Columbia material has a content of 0.0025%. In general, most of the British Columbia material has a bromine content higher than that of Manitoba material, in agreement with the proximity to the ocean of the source of this material. The results suggest that closely related species

have markedly different selective affinities for the element. To produce conclusive evidence of this, however, it would be necessary to grow plants under the same conditions.

The tables show definitely that different parts of the same plant have varying affinities for bromine. The green parts invariably have a higher bromine content than the roots of the same plant (*e.g.*, *Zea mays*, *Triticum vulgare*, *Brassica rapa*, *Trifolium pratense*, *Daucus carota*, *Pastinaca sativa* and *Solanum tuberosum*). In the case of *Zea mays* and *Triticum vulgare*, the fruits of which, as well as other parts of the plants, were analyzed, the fruit invariably contained the least amount of bromine. If this rule is generally applicable, it explains the low results for all cereal grains. In the two samples of *Triticum durum* grown in the same soil in 1933 and 1935, the 1935 sample has a bromine content of 0.0003%, that of 1933 a content of 0.0001%, suggesting a climatic factor.

Where comparisons are possible between the writer's figures and those of Damiens and Blaignon (24, 25), the former tend to be higher. The two species of melon examined by Damiens and Blaignon are unusually rich in bromine (0.00945 and 0.0262%). The writer has had as yet no opportunity to analyze similar material.

Marine Animals (Invertebrates)

Phylum Porifera

The four species of sponges examined were all non-calcareous single specimens. The writer has found no reference in the literature to the bromine content of Porifera.

The bromine content of the Pacific sponges examined is somewhat of the same order as the iodine content.

Phylum Coelenterata

The results obtained for the Pacific jellyfishes examined are remarkable for the fact that quite appreciable amounts of bromine are present, while iodine is present in negligible amount. For three of these coelenterates the water content had been determined (13), so that it is possible to calculate what proportion of bromine may be due directly to sea water (*cf.* Table XIV). The sea water from which these animals were taken contains about 4 mg. of bromine per 100 cc. (15).

TABLE XIV

Bromine in hydrozoa

Species	Bromine per 100 gm. fresh substance, %	Water per 100 gm., %	Bromine in equivalent weight of sea water, gm.
Aequorea forskalia	0.0062	94.6	0.0037
Aurelia flavidula	0.0121	93.7	0.0037
Matridium marginatum	0.0034	90.7	0.0036

Table XIV shows that only in the case of the sea anemone *Matridium marginatum* can all the bromine possibly be so accounted for. The table also stresses the different affinity for bromine possessed by the cells of these three species of Hydrozoa.

The *Verticillate fan coral* from Alaskan waters gave the highest value obtained for any material so far examined, 0.354% for the whole animal, and 0.876% for the skeleton (dried material).

Mendel (60) found no trace of bromine in *Gorgonia acerosa*. Cook (39) found none in the skeleton or coenenchyma of 10 species of *Gorgonacia*. The inability of these authors to demonstrate the presence of bromine is undoubtedly due to a faulty technique.

Quantitative observations have been made on corals by Mörner (66–68). The figures published are all given for the organic substance of the skeleton "Gorgonin", freed as far as possible from other tissues and from inorganic material. His figures may therefore be partially comparable with those given in this paper for the skeleton. Mörner carried out a large number of analyses on material collected in various parts of Europe and this continent. His results given in a condensed form, are, in percentages, (Gorgonaceae) Isidae, 0.74; Primnoidae, 2.94–3.76; Muriceidae, 1.18; Plexauridae, 0.96–4.20; Gorgonidae, 0.23–2.16; Gorgonellidae, 0.66–1.98; (Pematulaceae) various species, 0.97–1.89; (Antipathidae) two species, bromine absent; two species, 0.38 and 1.53.

From *Primnoa lepadifera*, Mörner was able to separate, by means of a baryta fractionation, a bromine-containing substance. This he subsequently identified as 3 : 5-dibromtyrosine.

Phylum Vermes, subphylum Annulata, class Chaetopoda, order Polychaeta. The annelid worm named by Dr. Cameron *Sabella columbiana* (14), was later identified as *Eudistylia gigantea* (70).

Dr. Cameron (14) makes the following statement as to the nature of the worm tubes examined.: "The *Diopatra* worm-tubes consist of an upper part, 4 to 6 inches in length, covered with shells and small Algae, and a lower part, up to 18 inches in length, of parchment-like consistency, consisting of concentric layers, the inner being translucent and usually perfect, the outer more or less damaged. The lower tube is secreted by the glands of Tori, the leathery upper tube in part is lip secretion. The tubes taken for examination were separated from adhering material (shells, Algae) and sand as far as possible, resolved into layers and air-dried. The *Chaetopterus* tubes had a similar structure to those of *Diopatra*. . . . The *Sabella (Eudistylia)* and *Bispira* tubes were tough, and horny in appearance, and consisted of numerous layers of translucent material. . . . . The *Phoronis* tubes were of thin hyaline material."

Only one sample of worm tissue has been examined. This contained 0.0195% of bromine. All the worm tubes examined contained bromine, the limits observed being 0.0019 and 0.319%. During the analyses it was

noticed that most of the material contained varying amounts of sand (*cf.* Cameron). Since no silica analyses have been made on this material, the necessary correction is not known.

The only observations on annelids in the literature seem to be those of Mörner (67), who found that the tubes of *Chaetopterus norwegicus* and *Hyalinaecia tubicola* contained, after careful removal of calcium carbonate, 0.18 and 0.12% of bromine, respectively.

Phylum Mollusca

The dermis of the foot of the sea whelk *Schizotherus nuttalli* is stated to be a secretion of the subdermis. In the two samples analyzed the figures (in each case for one or two specimens only) indicate that the bromine content is of the same order as the iodine content.

The byssus of *Mytilus* is an adhesive secretion, and the opercula of *Polynices* a protective secretion. The bromine content of these is considerably higher than the iodine content.

No pertinent data for *Mollusca* were found in the literature, but it is interesting to note that Friedländer (39) has shown that the colored substance of *Purpura* and *Murex* is a dibromindigo.

Phylum Chordata, subphylum Tunicata. The tests of only a few ascidians were examined. Bromine was an invariable constituent, the limits observed were 0.053 to 0.228%, there being notable variations in different species.

The literature contains nothing on the bromine content of tunicates.

Vertebrates

Numerous observers claim to have shown the presence of bromine in marked amounts in the thyroid and pituitary glands. These will be dealt with in turn, and subsequently figures available for body fluids and the remaining body tissues will be considered.

Thyroid

The writer has carried out a number of analyses of thyroid tissues of different animals, and of commercial thyroid preparations. Most of the thyroid material contains appreciable amounts of bromine; the limits observed are from a trace to 0.105%. In all cases where the iodine content was also determined it was considerably higher than the bromine content. The thyroid material from marine fishes has in most cases a bromine content higher than that of land mammals. This can probably be ascribed to diet.

Data are given in Table IX for several thyroglobulin preparations and one thyroid nucleoprotein preparation; all these were prepared by Dr. Cameron in 1926. In the case of thyroglobulin, the iodine content is decidedly high, while the bromine content appears to be of the same order as that of desiccated whole thyroid tissue. The thyroid nucleoprotein preparation contained none, or only a trace of bromine. To obtain some further clue to a possible function

of bromine in the thyroglobulin molecule, three lots of fresh thyroid glands were treated with 1% sodium chloride solution, and the bromine in the residue was determined. The results show no constancy in the proportion of bromine extracted, nor in the ratio of iodine extracted to bromine extracted; relatively more iodine than bromine was extracted.

A further experiment was carried out on beef thyroid. It was found, expressing the results in terms of 100 gm. of fresh material, that of the total bromine present (1.8 mg.), 1.7 mg. was extracted by 0.1 *M* sodium acetate, and 0.1 mg. was found in the residue. From the extract 2.22 gm. of impure thyroglobulin was obtained containing only 0.05 mg. of bromine, so that the remainder in the sodium acetate extract (1.65 mg.) was not present in thyroglobulin. Calculated to dry weights, the bromine percentages in thyroid and impure thyroglobulin were, respectively, 0.0078 and 0.0022. These experiments are not final but suggest that bromine is mainly associated with the non-thyroglobulin material of the thyroid.

Damiens (23) in a single analysis could detect no bromine in a dog thyroid. The analyses carried out by Bernhardt and Ucko (7), Lobat (55), and Serbescu and Buttu (82) indicated that appreciable amounts of bromine are present in dog, cattle and human thyroids. The writer's analyses of various species are in general agreement with theirs. The data of the other authors certainly are too high.

Of all the tissues examined, the thyroid appears to be the richest in bromine.

Pituitary

Bernhardt and Ucko (7) were the first to assert that considerable quantities of bromine are present in the pituitary. Zondek and Bier (100–105), using an even less accurate method of analysis, maintained that the anterior lobe of the pituitary contains a considerable amount, but that the posterior contains only traces. Jacobson (45) makes similar statements.

From the results obtained by them, H. Zondek and Bier (103) maintained that a bromine-containing endocrine principle is present in the anterior pituitary. They further state that they have isolated from the anterior pituitary a hydrolyzable substance containing bromine, which, when injected into dogs, produces signs of fatigue and asthenia. By analogy they supposed that the substance is tetrabromthyroxine. Such statements, if true, would obviously be important, and B. Zondek has even gone so far as to suggest the presence of a bromine hormone in the pituitary (99).

These findings, however, have been contradicted by Serbescu and Buttu (82), and Dixon (28). Dixon finds that the amount of bromine normally present in the pituitary is of the same order as that in blood, and that the Br/Cl ratio in the pituitary of hogs is, approximately, the same as that for other tissues. In a series of analyses of human tissues from both normal and pathological cases, Dixon obtained a high bromine content only for those cases where bromide was known to have been administered.

In Tables VII and VIII data are given of a few analyses carried out on rabbit, dog and beef pituitaries, and two pituitary preparations. The results obtained are in agreement with those of Dixon. The findings of Zondek and Bier, and others, that the pituitary plays an important role in the physiological function of bromine are almost certainly based on insufficient evidence.

Blood

The subject of bromine metabolism became especially interesting after Zondek (100-105) stated that blood bromine is lowered in manic-depressive states, and also in some organic cerebral diseases. These statements were supported by Sàcristan and Péraita (79), Klimke and Holthaus (48), Kuranami (51, 52), and others. However, Urechia and Retezeanu (89, 90), Charvat and Hejda (16) and Yates (98) found that results in various pathological conditions show very little constancy. Dixon (28) finds that blood bromine in manic-depressive psychotics, untreated with bromide or iodide for at least six months, exhibits the same type of variability as in normal subjects.

The normal bromine content of animal and human blood has been determined by a number of authors. The variations found are between 0.0004 and 0.0015 gm. per 100 cc. The writer's results for rat, rabbit and dog blood fall within these limits. Evidently the large variations encountered are due mainly to differences in intake.

Working with blood plasma and blood serum, Bernhardt and Ucko (7) obtained results lower than those for whole blood, while Urechia and Retezeanu (89, 90), and Guillaumin and Merejkowsky (40) obtained results higher than those for whole blood. According to Hastings, Harkins and Liu (44), the ratio, corpuscle Br/plasma Br, gives a smaller value than the corresponding ratio for chlorine, while Guillaumin and Merejkowsky obtain a higher value. Hastings and van Dyke (42, 43) ascribe this variation as being due to the pH of serum.

The writer has carried out analyses on two separate samples of beef blood obtained at a local abattoir. The amounts of bromine present were 0.00071 and 0.00133 gm. per 100 cc. A portion of the second sample was centrifuged (at 2,500 revolutions per minute), and the bromine content of the plasma determined (52% plasma and 48% corpuscles); 100 cc. of plasma was found to contain 0.00173 gm. of bromine, and 100 cc. of corpuscles 0.00090 gm. (calculated).

Certain authors have determined the amount of bromine present in organic combination in blood. Guillaumin and Merejkowsky (40) find that 63 to 88% of the bromine will not pass ultrafiltration. Ewer (30) states that 55 to 78% of the bromine is in organic combination.

Cerebrospinal Fluid

A number of authors have determined the bromine content of cerebrospinal fluid. The results obtained do not show any constancy. Rothschild and Malamud (78) have examined the bromine content of cerebrospinal fluid in certain psychoses without being able to deduce any conclusions.

Urine

Several bromine determinations were made by the present writer on the urine of normal individuals (morning samples taken before breakfast). The results obtained were 0.00113, 0.00181 and 0.00138 gm. per 100 cc. Though few, these results indicate that the amount of bromine in urine is not constant; presumably diet and concentration are the determining factors.

Tissues

In the analyses on the various tissues of the rat, rabbit, and dog reported in this paper, considerable variations in their bromine contents are noticeable. All the tissues analyzed contained bromine, ranging from a trace to 0.0106% (dry material).

Damiens (19, 22, 23), followed by Bernhardt and Ucko (7), and Dixon (28), have determined the bromine content of a large number of animal tissues. The values obtained by Damiens for dogs are of the same order as those obtained by Dixon and the present writer. The values obtained by Bernhardt and Ucko are somewhat higher. The determinations by Justus (46) are certainly too high.

In the writer's experiments the diets of the rats (bread, milk, and vegetables), the rabbits (hay and vegetables), and the dog (dog biscuit and water for two weeks) all contained traces of bromine (see Table X). Damiens and Blaignon (25), and Dixon (28), have shown that common salt, invariably a constituent of the diet, usually contains a small amount of bromine. This indicates the difficulties encountered in endeavoring to interpret results for the normal bromine content of tissues. Variations found in a normal series of analyses of animal tissues are undoubtedly very largely due to differences in bromine intake.

The Relation of Chlorine, Bromine and Iodine in Plant and Animal Tissues

The study of variations of the Br/Cl ratio in urine and blood has led to contradictory results. Frey (35–38) finds this relation constant after the administration of bromides. Appelmanns (3), on the other hand, finds that it is not constant; the elimination in urine may be deficient in bromine, or at times a predominance of the element occurs. Møller (64) criticizes Appelmanns' technique and supports Frey.

The theory of Nencki and Schoumov-Simanovsky (69) and Pflaumer (72), that after the administration of bromides, bromine replaces a certain amount of chlorine in blood and tissues, has been severely criticized by von Wyss (95–97) and others. It does not appear to have received confirmation in systematic studies of urinary elimination of bromides and of chlorides.

Frey finds the ratio of Cl/Br in blood and cerebrospinal fluid the same; in a number of pathological states, this ratio does not undergo great variations (Mishkis, Ritchie and Hastings (63)). Damiens (19, 22, 23) finds that the ratio Cl/Br is constant in blood and various organs. On the other hand,

Toxopeus (86, 87) finds that after the administration of chlorides and bromides, the distribution of bromine in the organism is quite different from that of chlorine.

Baldauf and Pincussen (4) find that the ratio Br/I in the blood in normal and various pathological cases varies from 97 to 105. Tanino (83, 84) finds that the ratio Br/I is higher in thyroid glands with a high colloid content than in those poor in colloid. The writer's findings on bromine distribution in the thyroid scarcely support this view.

The Distribution of Bromine in Inorganic Matter

At this stage it will be convenient to deal briefly with the present state of our knowledge regarding the distribution of bromine in inorganic matter. The data included, except where otherwise specified, are taken from Abegg (1) or Gmelin (39).

Bromine occurs in nature chiefly as bromides, and in solution as bromide ions. In the elementary composition of the earth (down to 10 km. depth) bromine occupies the 25th position (Vogt). The relative amount of bromine has been calculated as $1.10^{-3}\%$. The ratio of occurrence of the three halogens is Cl : Br : I = 250 : 1 : 0.1 (Vogt). Ackroyd (2) calculates the amount of bromine on land and in sea as being 0.000583 and 0.01–0.015% respectively. The solvent denudation for untold ages has eventually carried most of the bromine salts to the sea, which is its great receptacle (Vernadsky).

Numerous analyses have been carried out on the bromine content of sea water. The only recent analyses show, in gm. per 100 cc., for the Atlantic 0.0067 Br, to 20. 66 Cl (59), for the Adriatic 0.0064 Br to 18.38 Cl (93), and for the less saline Strait of Georgia, British Columbia, 0.0042 Br to 1.178 Cl at the surface, and 0.0056 Br to 1.559 Cl at a depth of 10 fathoms (15).

Bromine is present in varying amounts in mineral waters and salt beds (left by previous ocean waters). French mineral waters contain from traces to 8.2 gm. of bromine per litre; German mineral waters contain from none to 1.880 gm. of bromine per kg. Salt beds in Germany rich in Carnallite ($MgBr_2$, $KBr \cdot 6H_2O$) contain between 0.20 and 0.35% of bromine, those in Solikamsk, Russia, contain 0.17 to 0.30% of bromine (Efremov and Veselovskii (29)). On this continent appreciable amounts of bromine are found in the salt beds of Michigan, West Virginia, Ohio, and Pennsylvania. Various oil-bearing waters in Russia contain similar amounts (Maksimovich (57), Vinogradov (91)).

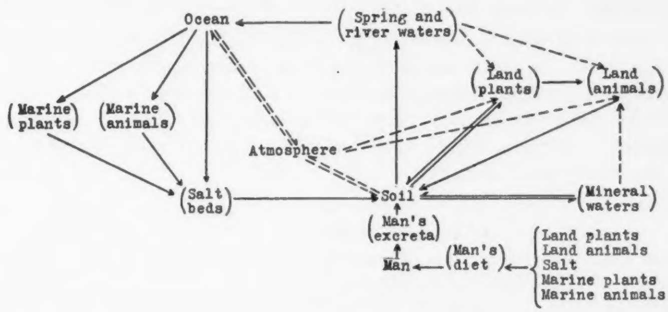
Bromine has been found as silver bromide in Chile, Mexico and France (Berthier), or as an isomorphic mixture of silver chloride-bromide (embolite) in Chile, Mexico, Honduras, and other localities. Occasionally it is found in the form of silver bromide-iodide.

The mother liquors of Chile saltpetre contain bromide and also a small amount of bromate (Grüneberg). According to Mitz, the bromate is formed from bromide by the action of micro-organisms.

The Bromine Cycle in Nature

The presence of bromine in marine plants and animals, land plants and animals, ocean waters, mineral waters, and in the salt beds left by previous ocean waters, has been given. From this it can be concluded that bromine is present in most soils, but to a varying degree. Since the large concentration of bromine in ocean waters is due to a constant denudation by the solvent action of waters, it must also be present (to a very small extent) in spring and river waters.

v. Fellenberg (31) has shown that soil and sea water will give up iodine to the atmosphere. Since the concentration of bromine in sea water is very much greater than that of iodine, it can be surmised that bromine is also present in the atmosphere. Taking all these data into consideration the following schematic arrangement of the bromine cycle in nature seems justified. In it the dotted lines represent possible, but unproved movements.



The Possible Physiological Significance of Bromine

The physiological significance of bromine has been discussed by a number of authors. Bertrand (10) suggests that bromine probably acts as a catalyzer in the body. Various assertions have been made by Ucko (88), Kuranami (51, 52), and others, that the anterior pituitary gland is concerned with bromine metabolism. Tanino (83, 84) and Jacobson (45) have considered a possible relation between the thyroid gland and bromine metabolism. They believe that the thyroid probably produces a bromine-containing substance, perhaps dibromthyroxine. Toxopeus (86, 87) maintains that the feeding of thyroid, thyroidectomy, and the injection of posterior pituitary extracts into animals, cause a change of blood bromine.

In reviewing the results that the present author has obtained with normal tissues, it is evident that, although the results for most tissues are virtually of the same order, thyroid and blood show in each case (rat, dog, and rabbit) a slightly higher value than the average figure for all tissues. The relatively high bromine content of blood obviously indicates that its presence in most tissues is merely dependent on their blood supply and is in itself of no particular significance.

The author has already discussed its relation to the pituitary gland, and his results confirm those of the more accurate workers, that its presence there is of no functional significance.

It is not yet possible to state that its presence in the thyroid is without significance, but the data recorded in this paper suggest that it is not functionally associated with the essential principle of the thyroid, so that further work will be necessary before any functional significance in relation to the gland can be stressed.

The review of the literature and the new results presented in this paper do not suggest any specific function for bromine in the living organisms.

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